BACTERIOLOGICAL CHARACTERIZATION OF FECES AND SOURCE DIFFERENTIATION

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BACTERIOLOGICAL CHARACTERIZATION OF FECES AND SOURCE DIFFERENTIATION

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Executive Summary

The potential for fecal pollution of Toronto Area Watersheds and lakefront is high due to the number of combined sewer and contaminated storm sewer outfalls plus a large animal and bird population in the Metro Toronto area. Efforts to solve pollution problems emphasize detection methods that can locate and differentiate the source of the pollution input (ie. human or non-human) and older methods, such as FC/FS ratios, are still being used. However, the variability of published information on FC/FS ratios and the bacterial populations in the feces of humans and other warm blooded animals presents a problem when trying to apply these findings to Microbiology Water Quality Studies, in Ontario. The majority of the studies used to develop bacteriological indices, such as the FC/FS ratio, have been conducted in other countries and therefore diet and other environmental factors as well as the media and methods used differ from those in Ontario. To remedy this existing situation, a 2 year study examining the bacterial levels and populations in the feces of humans, animals and birds was conducted during the period of June, 1984 to May, 1986 under the sponsorship of the Toronto Area Watershed Management Strategy (TAWMS) Study.

Fecal specimens were obtained from a wide variety of human and non-human sources likely to have an impact on the Metropolitan Toronto area. Both the concentrations and species distribution of the fecal coliform and fecal streptococci bacterial groups were determined using current Ministry of the Environment methods and newer procedures ie. m-TEC(IG) and m-ME that may soon be employed in Ontario. The effect of environmental stress on the fecal indicator levels, FC/FS ratios and bacterial populations from human and non-human fecal samples was

assessed along with a determination of the potential impact of pathogenic bacteria such as Salmonella spp., Pseudomonas aeruginosa and Campylobacter spp. Salmonella isolates were serotyped and tested for plasmid mediated antibiotic resistance.

The concentrations of fecal indicator bacteria were found to be high in all sources. Fecal coliform populations were comprised mainly of E. coli thus confirming the value of this organism as an indicator of fecal pollution. However, the insufficient heterogeneity of the FC populations present in the intestinal tracts of different animals limit the use of this group in source identification.

Problems with the specificity of some of the FS media along with the potential for other environmental sources of fecal streptococci suggest that these parameters are not as useful in indicating the presence of fecal pollution as E. coli, but may be valuable when run in conjunction with E. coli. Differences were found in the fecal streptococci populations isolated from humans and non-humans. These differences could be exploited in helping to identify such sources as livestock pollution but this would have to be accomplished close to the source of the inputs. The FC/FS ratios determined in this study indicate that the ratios proposed in 1969 by Geldreich are not applicable to current methods, as well, the application of environmental stress to fecal indicator bacteria altered relative populations and concentrations sufficiently to invalidate the use of FC/FS ratios unless applied close to the source of the pollution.

Pathogenic bacteria were found to be widespread in animals and especially in birds and this is of definite concern since many of those examined impact directly on the Toronto area environment. The isolation of multi resistant Salmonella serotypes from Gull feces poses a potential threat to human and livestock health as these birds are considered vectors in the spread of infection to both groups.

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1.0 INTRODUCTION

The extensive degradation of water quality in the Toronto area watersheds and lakefront, highlighted by the closure of a number of beaches due to fecal pollution has led to a desire to devise and implement corrective measures to reduce pollution loadings and improve water quality in this urban area. The same concerns have been expressed for other urban areas such as Ottawa and St. Catherines.

Current efforts to solve pollution problems have emphasized the importance of detection methods that are able not only to locate fecal pollution inputs and determine their impact; but also to differentiate between the source of pollution (ie.: human or non-human). The knowledge gained through such methods precedes the development of any management strategy plan to reduce pollution levels, since the control options implemented by such a plan would depend upon the original source of the pollution.

Fecal pollution of surface waters arising from any source is undesirable. The potential health hazard created by human fecal inputs is well documented. Outbreaks of typhoid fever, dysentery, cholera, hepatitis, poliomyelitis, gastroenteritis and ear and eye infections have been traced to human fecal contamination of surface waters (Dart and Stretton, 1980). Human inputs can come in the form of sanitary wastes from combined sewer outflows, waste treatment plant bypassing and illegal connections to storm sewers. When one considers the population of Metro Toronto and the size and age of its sewer system it can be seen that there is a great potential for human pollution of adjacent bodies of water.

Fecal wastes from the large animal and bird population in the Metro Toronto area may also contribute significantly to the deterioration of surface water quality. Their feces may be deposited directly into the water or gain entry through storm water runoff. Animals and birds have been implicated as vectors in the waterborne transmission of diseases to humans. Several studies (Benton, 1983; Khan, 1982; Smith et.al., 1977; Fennell & Morris, 1974; Houster, 1931, Skirrow and Benjamin, 1980) have shown gulls (Laridis canus and L. argentatus) to be the source of contamination to reservoirs and other water supplies and responsible for human outbreaks of gastroenteritis. As well, gulls have been reported as vectors in transmission of Salmonella infections to livestock (Fenlon, 1980; Williams et. al., 1977). Other avian sources such as pigeons (Columba livia), ducks (Anas platyrhynchos), and Canada geese (Branta canadensis) have been contributors to surface water contamination (Hill and Grimes, 1984; Palmer, 1983; Leuchtefeild et. al., 1980; Hussong et. al., 1979; Slandridge, 1979; Taylor et. al, 1979; Brierley et. al., 1975). These birds are also known to carry large numbers of pathogenic organisms in their feces. Carriage rates of Campylobacter jejunii among pigeons ranges between 20-90% (Fenlon, 1981), and wild ducks may have a Salmonella fecal excretion rate upwards of 4% (Reasoner, 1972).

Runoff from pastureland has also been shown to have a drastic effect on water quality causing considerable deterioration (Jawson et. al., 1982; Doran & Linn, 1979; Hollan et. al., 1972; Robbins et. al., 1972). The potential health risks to humans using waters downstream from these areas is significant because livestock are known to harbour many pathogens (Taylor, Brown & McDermott, 1982; Fenlon, 1980; Reasoner, 1972; Groves et. al., 1971; Robinson and Royal, 1971; Edel et. al., 1970).

Adding to this risk is the fact that strains of both pathogenic and indicator bacteria isolated from livestock feces show a high degree of antibiotic resistance possibly due to the addition of antibiotics to commercial feeds (Scott, 1984 personnel communication; Grabow et. al., 1973; Reasoner, 1972).

Other domestic animals ie. dogs and cats, can carry pathogenic bacteria and have been known to transmit diseases to humans (Fricker et. al., 1983; Khan, 1982 and 1970). Storm water runoff from streets, parks and fields may deposit fecal material from these animals directly into surface waters. A recent report on the Rideau River watershed suggested dog litter as a major contributor to pollution problems (Pitt, 1982).

Information relating to the differentiation of sources of fecal material comes mainly from past studies. A great degree of reliance has been placed on published literature which suggests a relationship between the source of fecal pollution and the fecal coliform to fecal streptococcus ratio (FC/FS) (Geldreich & Kenner, 1969). After studying the concentrations of fecal coliforms and fecal streptococci in human and non-human feces, Geldreich concluded that FC/FS ratios greater than 4 signified human wastes, while ratios less than 0.7 were indicative of non-human pollution. In the application of these ratios, the advice of the authors has often been ignored. They suggested that the ratio was most meaningful close to the source because its reliability decreased when the organisms were exposed to the water environment for more than 24 hours (Geldreich, 1972). They also indicated that the concentration of fecal indicator bacteria should indicate recent pollution input (ie. FS counts of 100orgs/100mls or greater) (Geldreich, 1976). Other fact-

ors which have been overlooked are that the media and methods used to develop the FC/FS ratios are not currently employed in Ontario. Geldreich developed the ratios using MPN methods for the determination of fecal coliforms and KF or PSE spread plates for fecal streptococci. The development of more selective media for the quantitation of E. coli and enterococci (Dufour, 1981) coupled with harsher recovery methods may drastically alter the FC/FS ratios obtained.

A number of other studies have produced results that conflict with those reported by Geldriech. They have reported a wide variety of FC/FS ratios among humans and non-humans and have shown that there can be considerable variation in the ratios obtained from members of the same species. A study of gull populations (Gould & Fletcher, 1978) demonstrated differences in the FC/FS ratios between species as well as within the same species. In addition, all of the birds sampled had FC/FS ratios considerably higher than 4. A study of Canada Geese and Whistling Swans (Hussong et. al., 1974) showed that environmental factors could effect the FC/FS ratios in the feces from these birds. In their 1979 study of humans and thirteen other warm blooded animals, Wheater, Mara, and Oragui reported ratios above 4 in a number of animals eg. dogs, ducks and gulls and a considerable amount of intra-species variability. The FC/FS ratios determined for individual humans ranged from 3 to 15,806. Their work also demonstrated that the use of different media could significantly effect the FC/FS ratios. Some workers (Feachem, 1975) suggest looking at changes in the FC/FS ratio by sampling various points both at and downstream from a pollution source. This method takes into account the die-off rates of the different bacteria found in humans and non-humans, a factor which is often overlooked as well. However, this procedure may not be feasible in an area where there are multiple inputs.

A determination of the species and biotypes making up the FS population in an impacted area has also been suggested as a way of distinguishing between human and non-human sources of fecal pollution. For instance, a number of workers have indicated that Streptococcus bovis is the predominant fecal streptococcus in the intestines of farm animals but is rare or absent in the human gut (Wheater et. al., 1979; Kenner, 1978; Geldriech, 1976; Medrile & Barnes, 1962). The organism has also been reported to be a significant part of the FS flora in dogs and cats (Wheater et. al., 1979; Kenner, 1978; Geldriech, 1976). The presence of this organism and the closely related S. equinus (found mainly in horse feces) in polluted waters would suggest recent input from non-human sources since both Streptococcus bovis and S. equinus do not survive long in the environment (Pitt, 1982). Some authors have also suggested that S. durans, S. faecium and certain S. faecalis biotypes are found mainly in non-human feces (Mundt, 1982; Kenner, 1978) however, other authors have not substantiated these findings (Wheater et. al., 1979).

The variability of published information on FC/FS ratios and bacterial populations in the feces of humans and other warm blooded animals presents a problem when trying to apply these findings to Ontario Microbiology Water Quality studies. The majority of these studies have been conducted in other countries and, therefore diet and other environmental factors may have a significant influence on the results. As well, the media and methods used in other parts of the world differ from those used in Ontario.

This study was designed to remedy the existing situation by examining the bacterial populations in the feces of humans, animals and birds likely to have an impact on the Metropolitan Toronto area. Both the

concentrations and species distribution of the FC and FS bacterial groups were determined using current MOE methods and newer procedures that may soon be employed in Ontario. The effect of environmental stress on the FC/FS ratios and the bacterial populations from the feces of human and non-humans were assessed along with a determination of the potential impact of pathogenic bacteria from these sources. By obtaining data directly relevant to Ontario it is hoped that the information will provide the means for a more concise interpretation of microbiological data obtained from surface water surveys and help in the identification of sources of fecal pollution. The ability to locate and identify sources makes the task of selecting appropriate pollution control options more feasible.

2.0 METHODS

2.1 Collection and Processing of Fecal Samples

Fecal samples were collected during the summer of 1984 from 12 sources: humans, gulls, ducks, geese, pigeons (Domestic), dogs, cats, chickens, pigs, cows, muskrats and raccoons. Some additional samples of various sources were taken during 1985. The results of these 1985 analyses are presented in some of the tables along with the 1984 results. The samples were obtained from the following sources:

Humans	- University of Toronto and MOE Personnel;
Gulls	- Humber West Park and Leslie St. Spit;
Geese	- Humber West Park;
Ducks	- Grenadier Pond
Pigeons	- commercial breeders and Queen's Park area;
Dogs	- U of T animal laboratories, Toronto Humane Society and privately owned dogs;
Cats	- U of T animal labs, Toronto Humane Society and privately owned cats;
Livestock (chickens, cows, pigs)	- Commercial farms in the Upper Humber River area;
Muskrats and Raccoons	- from animals trapped in or along the Humber River area.

Each sample was composed of feces from 3 or more individuals and was collected in sterile glass jars using sterile forceps or spatulas. To provide one human composite sample, 3 individual samples were collected in separate sterile jars and then combined to make the one sample.

A total of 24 grams of each sample was weighed out and transferred to sterile glass blender jars pre-calibrated to a 240ml volume. Sterile buffered phosphate water was added to the jar to the 240ml volume mark thus making a 1 in 10 dilution of the original fecal sample. The diluted samples were blended for 15 seconds at low speed to homogenize. Subsequent 10 fold dilutions of the blended samples were made in sterile dilution blanks containing 90mls of buffered phosphate water and these were used to carry out all the analyses.

2.2 Sample Analysis

Fecal coliform bacterial densities were determined by membrane filtration of appropriate dilutions of the fecal samples through Gelman GN6 47 mm cellulose nitrate filters with a porosity of 0.45μm. (Standard Methods for the Examination of Water and Wastewater, 1980). The filters were planted on m-TEC agar (Dufour, 1981) and incubated for 23 ± 1 hours at 44.5 ± 0.5°C. Both target and non-target colonies were counted. Target colonies were yellow, yellow-green and yellow-brown; non-target colonies were blue to blue-green in colour. To ensure the accuracy of the counts, only results

obtained from filters with target counts of between 10 and 100 colonies were used to calculate the fecal coliform density per gram of feces.

A second step for the determination of E. coli by urease treatment (Dufour, 1975) was incorporated into the m-TEC procedure. Filters with appropriate target counts (ie. between 10 and 100 target colonies) were removed from the m-TEC plates and placed on filter pads soaked in urea. The filters remained in contact with the urea for 15 minutes to allow for deaminization by non-E. coli coliform bacteria possessing urease. A second count of all urease negative colonies (all yellow, yellow-green and yellow-brown colonies) was taken.

A single-step method for the determination of E. coli using m-TEC with indoxyl-β-D-glucoside (IG) (Dufour, 1979) was also employed. The method of analysis, incubation temperature and time and the counting procedures were similar to those used in conjunction with regular m-TEC. However, the second step E. coli determination involving the transfer of filters to urea was not necessary because of the addition of IG. This chromogenic analogue of cellobiose is catabolized to glucose and indoxyl by non-E. coli coliform bacteria processing β-D-glucosidase. The degradation of IG produces blue haloed yellow to yellow-green colonies which can be distinguished from regular yellow, yellow-green and yellow-brown target colonies.

A total of 10 target and non-target colonies were picked from

both m-TEC and m-TEC IG for each of the fecal samples. These were streaked on Nutrient Gelatin with Yeast Extract agar (NGYE) for purity. Pure isolates were preserved at -70°C in a 40% glycerol solution until they could be identified. Biochemical identification of the isolates was by API 20E identification strips for Enterobacteriaceae.

Stressed fecal samples were analysed in the above manor with the exception that bacterial isolates were not picked and identified.

Analysis of the fecal samples for fecal streptococci and Enterococci was also by membrane filtration. The filters were planted on two media for the recovery of fecal streptococci: m-Enterococcus agar (Difco) (Slanetz and Barkley, 1957) and KF (Difco) modified by the addition of a 0.5% starch agar overlay (G. Palmateer personnel communication, 1983) for the differentiation of S. bovis colonies. Both media were incubated for 48 hours at 35°C. Upper and lower counting limits of 10-150 target organisms were applied to the reported results. After counting, the modified KF plates with the starch agar overlay were flooded with a solution of grams iodine. A second count of all starch hydrolzing colonies (colonies within clear zones) was taken and recorded as the S. bovis count.

Enterococci were recovered on m-ME agar (Dufour, 1980) which also contains indoxyl-β-D-glucoside. The m-ME plates were incubated for 48 hours at 41.5 ± 0.5°C. The incubation time was modified from the original 24 hours suggested by Dufour

as it allowed for a slight increase in recovery of target organisms. The addition of IG to the medium facilitates differentiation of the ß-D-glucosidase Enterococci from other fecal streptococci. Both target and non-target colonies were enumerated. Target colonies on m-ME were purple, white-blue to dark blue with blue haloes from degradation of the IG. Non-targets were pink to maroon non-haloed colonies.

A total of 20 isolates both target and non-target were picked from each of the 3 media for each fecal sample and streaked on Brain Heart Infusion agar (BHI) for purity. Pure isolates were also preserved at -70°C in a 40% gycerol solution until they could be identified by a biochemical and serological testing scheme (See Appendix A).

Stressed samples were analysed using the above procedures. A total of 10 isolates (target and non-target) were picked from m-Enterococcus and m-ME agars and 20 isolates from modified KF agar for each stressed sample. These were also streaked to BHI and preserved for identification later on.

Determination of the densities of pathogenic bacteria in feces was accomplished by most probable number (M.P.N.) techniques (Standard Methods For the Examination of Water and Wastewater, 1980).

Recovery of Pseudomonas aeruginosa was facilitated by pre-enrichment in 1% buffered peptone water (Standard Methods, 1980). A 3x5 M.P.N. matrix of buffered peptone water was inoculated with appropriate dilutions of the samples and

incubated for 24 hours at 41.5 ± 0.5°C. The samples were then enriched in Drakes basil medium (Drake, 1966) modified by the addition of the following antibiotics: sulphapyridine 0.176 g/L, Kanamycin sulphate 0.0085 g/L, Naladixic acid 0.037 g/L and Actidione 0.150 g/L. Incubation of the enrichment tubes was for 48 hours at 41.5 ± 0.5°C at which time the tubes were checked for fluorescent properties under UV light. Growth from fluorescing tubes was subcultured to skim milk agar (Brown and Foster, 1969), incubated at 35°C for up to 3 days and checked daily for fluorescence, production of pyocyanin pigment, reduction of casein and for a characteristic grape-like odour. Presumptive positive cultures of P. aeruginosa were confirmed by inoculation into acetamide agar slants. Stressed samples were analysed for P. aeruginosa in a identical manor.

Salmonella spp. were quantitatively isolated from the fecal samples by pre-enrichment in 1% buffered peptone water using the same 3x5 MPN setup that was used for Pseudomonas aeruginosa. Rappaport's Tethathionate Broth (Rappaport et. al., 1956) coupled with 42°C incubation was used in the enrichment phase and the tubes were subcultured at 48 hours to duplicate plates of Xylose, Lysine, Desoxycholate agar (Taylor, 1965) and Brilliant Green agar (Difco) modified by the addition of 0.1% Sulfonamide. Cultures on the solid media were incubated for 24 hours at 37°C and checked for growth of presumptive Salmonella colonies. A minimum of 3 presumptive colonies per plate from each medium were picked and streaked to NGYE agar for purity. Pure isolates were identified by API 20E biochemical identification strips are

preserved until they could be identified. Some of the confirmed isolates of Salmonella spp. were sent to the Ontario Ministry of Health Enteric Reference laboratory for serological identification. These identified serotypes were tested for their antibiotic resistance. Stressed samples were not analysed for Salmonella.

Quantitative analysis of the fecal samples for Campylobacter spp. was performed using a 3x5 M.P.N. matrix with Preston's Campylobacter. Selective broth (Oxoid) (Bolton et. al., 1983) as the enrichment medium. The broth cultures were incubated microaerophilically in anaerobic jars using Oxoid Campylobacter gas packs for 24 hours at 42°C and subcultured to Preston's Campylobacter Selective Agar (Oxoid) (Bolton et. al., 1983). The plates were also incubated microaerophilically at 42°C and examined at 24 and 48 hours for presumptive growth of Campylobacter colonies. Presumptive isolates were picked and streaked on Trypticase Soy agar with Oxoid Campylobacter Growth supplement for purity. Pure isolates were gram stained and tested for cytochrome oxidase and catalase. All gram negative gull-shaped or spiral rods that were oxidase and catalase positive were preserved and identified latter using the biochemical identification procedure of Karmali and Skirrow (1985).

Note: During the fall of 1985, samples from grass and other vegetation growing in the vicinity of the Humber River/Black Creek area were analysed for fecal streptococci. The recovery methods and results of these analyses are presented in APPENDIX B.

3.0 RESULTS

The results of the study are presented in tabular form (Tables 1 to 19). The concentrations of fecal bacteria in feces are presented in Tables 1 to 10 while Tables 11 to 17 document the species breakdown of indicator and pathogenic bacteria in feces. The bacterial concentrations are all reported per one gram of feces (wet weight) and the species percentages are calculated from the total number of isolates identified from each source.

3.1 Geometric Mean Levels of Fecal Coliforms, Fecal Streptococci, E. Coli and Enterococci and the Fecal Coliform to Fecal Streptococcus Ratios in Human and Non-Human Feces (Table 1).

The concentrations of fecal coliform bacteria in both human and non-human feces is high ranging from 10^5 to 10^8 with the exception of horses, which have a somewhat lower concentration of 8.7×10^4. Levels of E. coli as determined by the two methods urease and m-TEC IG are in fairly good agreement with each other and with the fecal coliform results, however, the m-TEC IG reported results tend to be somewhat higher than the urease results and in some cases higher than the fecal coliform results. Most of the avian samples (ie. pigeons (wild), gulls, ducks, geese and chickens) show higher fecal coliform and E. coli levels than do human samples. Whereas dogs exhibit fecal coliform and E. coli levels similar to humans.

Fecal streptococcus concentrations are lower in humans (10^5) than in most non-human samples (10^6 to 10^8) but the lowest concentration concentration ($4x10^5$) is reported in cat feces. Raccoons show the highest concentration at $2x10^8$. Concentrations of fecal streptococci recovered on KF tend to be higher than those on m-Enterococcus, with the exception of geese, muskrat and cow fecal samples which show lower recoveries on KF. Recoveries of enterococci on m-ME are lower than the fecal streptococcus concentrations recovered on either KF or m-Enterococcus. However, the m-ME results from pigeon (domestic & wild) feces are higher than m-Enterococcus and the ME results from dog feces are higher than both the KF and m-Ent result. Overall there are no significantly large differences reported between the three media.

The fecal coliform to fecal streptococcus ratios FC/FS are determined using the m-TEC and m-Enterococcus results. The highest reported ratio is from Humans at 27. Several non-human samples also showed high ratios (ie. gulls, ducks, wild pigeons and dogs). One half of the 14 non-human samples tested exhibited ratios above 4 and in some cases (ie. gull and wild pigeons) similar results to humans are reported. Only 3 of the 14 non-human samples tested had ratios below 0.7.

3.2 Comparison of Fecal Coliforms (E. Coli) to Fecal Streptococci (Enterococci) Ratios Using Different Media. (Table 1B).

A comparison of ratios of fecal coliforms (m-TEC) to the various streptococci (m-ENT & KF) and Enterococci (m-ME) media shows that very little difference in the ratios may be

achieved using these different combinations. However, combinations of E. coli (urease) with KF or m-ME demonstrate a somewhat greater difference in the ratios between sources. Only 3 non-human sources had ratios above 10 as opposed to 4 sources using the standard m-TEC/m-Enterococcus ratio. In addition, the spread between human and non-human ratios increases. The combination of E. coli (urease) with m-Enterococcus did not show as great a difference as the other streptococcus media.

Use of E. coli (m-TEC IG) with m-Enterococcus, KF and m-ME resulted in some differences in the reported ratios but these were not as evident as the E. coli (urease)/KF or E. coli (urease)/m-ME results.

3.3 Levels of Pathogenic Bacteria in Human and Non-human Feces (Table 2).

Pathogenic microorganisms are recovered in the feces of all but one of the samples tested. Both gulls and ducks showed fairly high levels of all three of the investigated pathogens, with gulls having the highest levels of Pseudomonas aeruginosa (13.4/gram) and ducks, the highest levels of Salmonella spp. (19.9/gram). Turkeys exhibited the highest levels of Campylobacter spp. (93/gram) with wild pigeons having the second highest reported levels (7.85/gram).

TABLE 1A: Geometric Mean Levels of Fecal Coliforms, *E. coli*, Fecal Streptococci and Enterococci and the Fecal Coliform to Fecal Streptococcus Ratios in Human and Non-Human Feces Sampled During the Summer of 1984

FECAL INDICATOR BACTERIA PER GRAM OF FECES

SAMPLE SOURCE	FECAL COLIFORMS M-TEC	E. COLI UREASE	E. COLI M-TEC IG	FECAL STREPTOCOCCI M-ENT	FECAL STREPTOCOCCI KF	ENTEROCOCCI M-ME	F. COLIFORM/ F. STREPTOCOCCUS RATIO M-TEC/M-ENT
Humans	$2.6x10^7$	$2.6x10^7$	$2.2x10^7$	$9.5x10^5$	$1.0x10^6$	$7.9x10^5$	27.0
Gulls	$1.85x10^8$	$1.35x10^8$	$1.55x10^8$	$8.6x10^6$	$8.7x10^6$	$7.7x10^6$	21.5
Ducks	$1.5x10^8$	$1.5x10^8$	$1.8x10^8$	$8.4x10^6$	$9.4x10^6$	$5.6x10^6$	17.8
Geese	$7.8x10^6$	$7.8x10^6$	$3.6x10^6$	$1.0x10^7$	$5.1x10^6$	$9.7x10^6$	0.8
Pigeons (Domestic)	$1.3x10^8$	$1.3x10^8$	$2.9x10^8$	$3.0x10^7$	$1.2x10^8$	$4.5x10^7$	4.4
Pigeons + (wild)	$3.2x10^8$	$3.2x10^8$	NA	$1.4x10^7$	$1.7x10^7$	$1.6x10^7$	23.0
Dogs	$2.3x10^7$	$2.3x10^7$	$2.4x10^7$	$1.8x10^6$	$2.9x10^6$	$3.2x10^6$	13.0
Cats	$5.9x10^5$	$4.0x10^5$	$6.5x10^5$	$4.0x10^5$	$2.6x10^6$	$3.9x10^5$	1.5
Chickens	$9.9x10^7$	$9.6x10^7$	$6.55x10^7$	$3.8x10^7$	$9.4x10^7$	$2.0x10^7$	2.6
Pigs	$1.0x10^7$	$9.9x10^6$	$9.0x10^6$	$5.85x10^6$	$8.2x10^6$	$1.75x10^6$	1.7
Cows	$5.0x10^5$	$5.0x10^5$	$6.6x10^5$	$8.8x10^6$	$7.5x10^6$	$1.5x10^6$	0.06
Horses °+	$8.65x10^4$	$7.2x10^4$	NA	$2.1x10^7$	NA	NA	0.004
Turkeys°+	$2.1x10^7$	$2.1x10^7$	NA	$4.4x10^6$	NA	NA	4.8
Muskrats	$3.6x10^6$	$3.4x10^6$	$3.5x10^6$	$9.0x10^5$	$7.8x10^5$	$9.55x10^5$	4.0
Racoons°	$6.5x10^7$	$6.2x10^7$	$7.7x10^7$	$2.0x10^8$	$5.8x10^8$	$2.5x10^8$	0.32

° less than 3 samples analysed
+ 1985 Data
* Approximate values

NA - not analysed

TABLE 1B: Comparison of Fecal Coliforms (E. coli) to Fecal Streptococci (Ent.) Ratios Using Different Media Studied

	FC/FS Ent	FC/FS KF	FC/Ent ME	EC ur/FS Ent	EC ur/FS KF	EC ur/Ent ME	EC IG/FS Ent	EG IG/FS KF	EC IG/Ent ME
Humans	27.4	26.0	32.9	27.4	26.0	32.9	23.2	22.0	27.8
Gulls	21.5	21.3	24.0	15.7	15.5	17.5	18.0	17.8	20.1
Ducks	17.9	16.0	26.8	17.9	16.0	26.8	21.4	19.1	32.1
Geese	0.8	1.5	0.8	0.8	1.5	0.8	0.4	0.7	0.4
Pigeons (Domestic)	4.3	1.1	2.9	4.3	1.1	2.9	9.7	2.4	6.4
Pigeons (Wild)	22.9	18.2	20.0	22.9	18.2	20.0	NA	NA	NA
Dogs	12.8	7.9	7.2	12.8	7.9	7.2	13.3	8.3	7.5
Cats	1.5	0.2	1.5	1.0	0.2	1.0	1.6	0.3	1.7
Chickens	2.6	1.1	5.0	2.5	1.0	4.8	1.7	0.7	3.3
Pigs	1.7	1.2	5.7	1.7	1.2	5.7	1.5	1.1	5.1
Cows	0.06	0.07	0.3	0.06	0.07	0.3	0.08	0.09	0.4
Horses	0.004	NA	NA	0.003	NA	NA	NA	NA	NA
Turkeys	4.8	NA	NA	4.8	NA	NA	NA	NA	NA
Muskrats	4.0	4.6	3.8	3.8	4.4	3.6	3.9	4.5	3.7
Racoons	0.3	0.1	0.3	0.3	0.1	0.2	0.4	0.1	0.3

TABLE 2: Geometric Mean Levels of Enteric Pathogens in Human and Non-Human Feces Sampled During the Summer of 1984

SAMPLE SOURCE	PATHOGENS PER GRAM OF FECES		
	Pseudomonas aeruginosa	Salmonella spp.	Campylobacter spp.
Humans	0.6*	<0.3	<0.3
Gulls	13.4	1.8*	2.6
Ducks	1.9	19.9*	1.3*
Geese	<0.3	0.5*	2.3*
Pigeons (Domestic)	0.4*	<0.3	<0.3
Pigeons + (wild)	<0.3	<0.3	7.85
Dogs	0.7*	<0.3	0.6*
Cats	<0.3	<0.3	0.405*
Chickens	0.3	1.1*	2.1
Pigs	<0.3	<0.3	4.4*
Cows	<0.3	<0.3	0.6*
Horses °+	NA	<0.3	<0.3
Turkeys°+	NA	NA	93.0
Muskrats	0.3	<0.3	<0.3
Racoons°	0.4*	0.5*	<0.3

+ 1985 Data
° less than 3 samples analysed

* - Approximate values
NA - not analysed

3.4 Comparison of the Levels of Fecal Coliforms, E. Coli, Fecal Streptococci and the Fecal Coliform to Fecal Streptococci Ratios in Human Feces (Table 3).

A breakdown of Human fecal samples according to the sex, age and diet of the Host shows the amount of variation that can occur within the Human population. It would appear that the consumption of garlic has a tendency to lower the fecal streptococcus concentrations and thus increase the FC/FS ration. Overall, it can be seen that the FC/FS ratio may fluctuate from less than 1 to over 2000.

3.5 Comparison of the Geometric Mean Levels and Individual Levels of Indicator and Pathogenic Bacteria and the FC/FS Ratios in Gull Feces Isolated from Leslie St. Spit and Humber West Park 1984-1985 (Tables 4A, 4B and 4C).

Table 4A shows the Geometric Mean concentrations from combined 1984 and 1985 data from both the Leslie St. Spit and Humber West Park. The FC/FS ratio of the combined data is much lower than the 1984 result alone, dropping from 21.5 to 2.2. This is due to the increase in fecal Streptococcus concentrations from 1984 to 1985 (8.6×10^6 to 6.2×10^7). Table 4B is a breakdown of Table 4A and demonstrates the differences that can occur in the Geometric mean levels both spatially and temporarily. It can be seen that there is considerable variability in the FC/FS ratios from location to location and from year to year. Table 4C is a further breakdown of Table 4B and documents the variation that can occur

TABLE 3: A Comparison of the Levels of Fecal Coliforms, E. coli, Fecal Streptococci and the Fecal Coliform to Fecal Streptococci Ratio in Human Feces Sampled During 1984 & 1985.

DATE OF SAMPLE	SAMPLE SOURCE	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO
July 15/84	3 Male Technicians	4.2×10^6	4.1×10^6	1.1×10^6	3.8
July 27/84	3 Female Technicians +	2.3×10^8	2.3×10^8	3.1×10^5	742.0
Aug. 26/84	3 Male & Female Tech.	1.8×10^7	1.8×10^7	2.6×10^6	6.9
Sept. 24/84	Female 40-45 Yrs.	1.7×10^7	1.7×10^7	5.3×10^7	0.3
Sept. 24/84	Male 45-50 Yrs.	4.0×10^6	4.0×10^6	1.6×10^6	2.5
Oct. 10/84	Female 25-30 Yrs.	2.0×10^8	2.0×10^7	9.5×10^5	207.0
Feb. 11/84	Male 40-45 Yrs.	3.0×10^7	3.0×10^7	5.1×10^7	0.5
Feb. 18/85	Female 25-30 Yrs.	1.0×10^7	1.0×10^7	1.0×10^5	100.0
Feb. 18/85	Male 25-30 Yrs.	1.3×10^7	2.5×10^7	2.5×10^7	0.5
Geometric Mean		(2.1×10^7)	(2.1×10^7)	(2.75×10^6)	(7.6)
Oct. 1/84	Female 40-45 Yrs. (Garlic consumed)	1.3×10^6	1.3×10^6	3.0×10^4	43.3
Oct. 22/84	Female 40-45 Yrs. (Garlic consumed)	2.1×10^7	2.1×10^7	1.0×10^4	2100.0
Oct. 15/84	Male 45-50 Yrs. (Garlic consumed)	4.1×10^6	4.1×10^6	1.1×10^7*	0.37
Oct. 30/84	Female 40-45 Yrs. (Garlic consumed)	3.2×10^7	3.2×10^7	9.7×10^5	33.7
Nov. 4/84	Male 20-25 Yrs. (Garlic consumed)	1.6×10^7	1.6×10^7	7.0×10^5	22.8
Jan. 21/85	Male 45-50 Yrs. (Garlic consumed)	5.6×10^6	5.6×10^6	2.1×10^4	267.0
Feb. 17/85	Female 25-30 Yrs. (Garlic consumed)	6.6×10^7	6.6×10^7	1.7×10^7	3.9
Geometric Means		(1.1×10^7)	(1.1×10^7)	(3.6×10^5)	(30.5)
Geometric Means All Samples		(1.6×10^7)	(1.6×10^7)	(1.1×10^6)	(14.5)

+ one person had consumed Garlic previous to the sampling

* most of the streptococci recovered were non-Enterococci species

TABLE 4A: Geometric Mean Levels of Fecal Coliforms, E. coli and Fecal Streptococci from Gull Feces Sampled at Leslie St. Spit and Humber West Park, 1984-1985.

FECAL COLIFORMS	E. COLI	FECAL STREPTOCOCCI	FC/FS RATIO
1.3×10^8	8.9×10^7	6.2×10^7	2.2

All counts reported per one gram of feces

TABLE 4B: A Comparison of the Geometric Mean Levels of Fecal Coliforms, E. coli, Fecal Streptococci and the Fecal Coliform to Fecal Streptococcus Ratio from Gull Feces Sampled at Leslie St. Spit and Humber West Park.

LOCATION/DATE	FECAL COLIFORMS	E. COLI	FECAL STREPTOCOCCI	FC/FS RATIO
Leslie St. Spit 1984	2.4×10^8	1.9×10^8	2.8×10^7	8.6
Leslie St. Spit 1985	6.1×10^7	5.6×10^7	4.8×10^7	1.3
Humber West Park 1984	1.85×10^8	1.35×10^8	8.6×10^6	21.5
Humber West Park 1985	1.2×10^8	4.3×10^7	1.25×10^9	0.093

All counts reported per one gram of feces.

TABLE 4C: A Comparison of the Levels of Fecal Coliforms, E. coli, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococci Ratios from Gull (Larus spp.) Feces Sampled at Leslie St. Spit and Humber West Park, 1984-1985.

LOCATION AND SAMPLING DATE	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO	SALMONELLA PER 1 GRAM FECES	PSEUDOMONAS PER 1 GRAM FECES	CAMPYLOBACTER PER 1 GRAM FECES
Leslie St. + Spit July 16/84	4.3×10^8 1.2×10^8 1.0×10^8	4.3×10^8 7.0×10^7 7.5×10^7	3.3×10^7 1.9×10^7 3.5×10^7	13 6.4 3.0	NA NA NA	>11.0 1.15 11.0	NA NA NA
Leslie St. Spit July 11/85	2.6×10^7 2.1×10^8 4.2×10^7	2.6×10^7 2.1×10^8 3.2×10^7	5.7×10^7 1.1×10^8 1.8×10^7	0.5 1.9 2.3	NA NA NA	NA NA NA	3.5 3.5 3.5
Humber W.P. June 10/84 June 17/84 Aug. 6/84 Aug. 12/84	 1.8×10^8 2.5×10^8 1.4×10^8 4.0×10^7	 1.7×10^8 1.1×10^8 1.4×10^8 3.0×10^7	 9.2×10^6 3.0×10^6 2.3×10^7 9.0×10^6	 19.3 84.7 6.1 4.4	 <0.3 9.3 2.1 NA	 3.5 46 15 NA	 Present Present 4.2 1.6
Humber W.P. June 10/85	4.3×10^7 1.45×10^8 2.5×10^8	2.7×10^7 3.3×10^7 8.9×10^7	1.0×10^9 1.2×10^{10} 1.6×10^8	0.04 0.01 1.6	NA NA NA	NA NA NA	NA NA NA

+ Analysis performed by the Ont. Min. of the Environment

within the gull species during the same sampling period and location. Variations in the concentrations of pathogenic bacteria are also evident.

3.6 Comparison of the Levels of Fecal Coliforms, E. Coli, Fecal Streptococci and Pathogens, and the Fecal Coliform to Fecal Streptococcus Ratios in Goose, Feces (Table 5).

Although there is some variation in the levels of fecal indicator bacteria, the fecal coliform to fecal streptococcus ratios tend to remain consistently below 4 in goose feces with most of the reported ratios falling at or below 0.7. There is little variation in the levels of pathogenic bacteria isolated from geese and all the reported counts are low. The one exception to this is the levels of Campylobacter spp. which tends to fluctuate over a wide range (<0.3 to >110/per 1 gram of feces).

3.7 Comparison of the Levels of Fecal Coliforms, E. Coli, Fecal Streptococci, and Enteric Pathogens and the Fecal Coliform to Fecal Streptococcus Ratios in Domestic and Wild Pigeons (Table 6).

The levels of fecal coliform bacteria and E. coli tend to be somewhat higher in wild pigeons and the concentrations of fecal streptococci are lower. This would account for the increased fecal coliform to fecal streptococcus ratios exhibited by these birds. As well, the wild birds tend to harbour consistently high levels of Campylobacter spp. in their intestinal tracts, but do not carry Pseudomonas aeruginosa.

TABLE 5: A Comparison of the Levels of Fecal Coliforms, *E. coli*, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococci Ratio in Goose (Branta Canadensis) Feces Sampled at Humber West Park 1984-1985.

DATE OF SAMPLING	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO	SALMONELLA PER 1 GRAM FECES	PSEUDOMONAS PER 1 GRAM FECES	CAMPYLOBACTER PER 1 GRAM FECES
June 24 1984	9.9×10^6	9.9×10^6	1.8×10^7	0.55	<0.3	<0.3	0.4
July 22 1984	6.0×10^6	6.0×10^6	1.9×10^7	0.3	1.5	<0.3	<0.3
July 29 + 1984	1×10^4	1×10^4	1.2×10^6	0.008	<0.3	<0.3	<0.3
Aug. 12 1984	8.1×10^6	8.0×10^6	2.9×10^6	2.8	<0.3	<0.3	>110
Aug. 11 1985	1.7×10^4	1.5×10^4	2.7×10^4	0.6	<0.3	<0.3	4.3
Geometric Means	1.7×10^6	1.6×10^6	2.3×10^6	0.7	0.45*	<0.3	2.7*

\+ Sample was dry - not included in Geometric means
* Approximate value

TABLE 6: A Comparison of the Levels of Fecal Coliforms, *E. coli*, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococci Ratio in the Feces of both Wild and Domestic Pigeons Sampled During 1984 and 1985.

DATE OF SAMPLING	SOURCE OF SAMPLE	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO	SALMONELLA PER 1 GRAM FECES	PSEUDOMONAS PER 1 GRAM FECES	CAMPYLOBACTER PER 1 GRAM FECES
July 2 1984	Breeder (Domestic)	3.7×10^8	3.7×10^8	1.0×10^8	3.7	<0.3	<0.3	<0.3
July 22 1984	Breeder (Domestic)	3.5×10^7	3.5×10^7	2.7×10^7	1.3	<0.3	0.9	<0.3
July 29 1984	Breeder (Domestic)	1.8×10^8	1.8×10^8	9.9×10^6	18.2	<0.3	<0.3	<0.3
Geometric Mean		(1.3×10^8)	(1.3×10^8)	(3.0×10^7)	(4.4)	(<0.3)	(0.44)*	(<0.3)
June 24 1985	Queen's Park (wild)	1.8×10^8	1.8×10^8	8.7×10^6	20.7	<0.3	<0.3	7.85
July 15 1985	Queen's Park (wild)	5.0×10^8	5.0×10^8	1.7×10^7	29.4	<0.3	<0.3	7.85
July 15 1985	Philosopher's Walk (wild)	3.7×10^8	3.7×10^8	1.8×10^7	20.5	<0.3	<0.3	7.85
Geometric Mean		(3.2×10^8)	(3.2×10^8)	(1.4×10^7)	(22.9)	(<0.3)	(<0.3)	(7.85)

*Approximate Value

3.8 Comparison of the Levels of Fecal Coliforms, E. Coli, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococcus Ratios in Dog Feces (Table 7).

There is some variability exhibited in the indicator bacterial levels within the species and the fecal coliform to fecal streptococcus ratios tend to fluctuate greatly ranging from 0.015 to 174. The levels of pathogenic bacteria isolated is quite low and pathogens tend to be isolated somewhat more frequently from kennelled animals.

3.9 A Comparison of the Levels of Fecal Coliforms, E. Coli, Fecal Streptococci and Enteric Pathogens, and the Fecal Coliform to Fecal Streptococcus Ratios in Cat Feces (Table 8).

As with dogs, there is some variability in the fecal indicator levels in cats, although the concentrations reported are much lower than those found in dog feces. The fecal coliform to fecal Streptococcus ratios exhibit less fluctuation ranging only from 0.2 to 4.4 per gram of feces. Again there is a tendency to isolate pathogenic bacteria more often from kennelled animals however, the concentrations are still quite low.

3.10 Geometric Mean Levels of Fecal Coliforms, E. Coli, Fecal Streptococci, Enterococci and Pseudomonas Aerugionosa as well as Fecal Coliform to Fecal Streptococcus Ratios in Stressed Fecal Samples (Table 9).

TABLE 7: A Comparison of the Levels of Fecal Coliforms, E. coli, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococcus Ratio in Dog Feces Sampled During 1984 and 1985.

DATE OF SAMPLING + LOCATIONS	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO	SALMONELLA PER 1 GRAM FECES	PSEUDOMONAS PER 1 GRAM FECES	CAMPYLOBACTER PER 1 GRAM FECES
July 2/84 Tor.Hum.Soc.	2.1×10^7	2.1×10^7	$>1.5 \times 10^8$	0.2*	<0.3	<0.30	1.5
July 22/84 Tor.Hum.Soc.	1.4×10^8	1.4×10^8	1.1×10^6	127	<0.3	4.3	<0.3
Aug. 6/84 Suburban	6.1×10^7	6.1×10^7	3.5×10^5	174	<0.3	<0.3	<0.3
Aug. 19/84 U. of T. Labs	2.4×10^7	2.4×10^7	9.8×10^5	24.5	NA	NA	NA
Aug. 29/84 U. of T. Labs	NA	NA	NA	NA	NA	NA	0.9
Oct. 4/84 Rural	1.4×10^6	1.4×10^6	2.6×10^7	0.05	NA	NA	NA
July 27/85 Rural	5.7×10^7	5.3×10^7	6.1×10^5	93.4	NA	NA	NA
Aug. 6/85 Suburban	8.0×10^4	8.0×10^4	5.2×10^6	0.015	<0.3	<0.3	0.6
Sept. 19/85 Rural	4.9×10^8	4.9×10^8	2.1×10^7	23.3	<0.3	<0.3	<0.3
Geometric Mean	1.8×10^7	1.8×10^7	4.2×10^6*	4.3*	<0.3	0.5	0.5

* Appfoximate Value

TABLE 8: A Comparison of the Levels of Fecal Coliforms, E. coli, Fecal Streptococci and Enteric Pathogens and the Fecal Coliform to Fecal Streptococcus Ratio in Cat Feces Sampled During 1984 and 1985.

DATE OF SAMPLING + LOCATIONS	F. COLIFORMS PER 1 GRAM FECES	E. COLI PER 1 GRAM FECES	F. STREPTOCOCCI PER 1 GRAM FECES	FC/FS RATIO	SALMONELLA PER 1 GRAM FECES	PSEUDOMONAS PER 1 GRAM FECES	CAMPYLOBACTER PER 1 GRAM FECES
June 24/84 Suburban	1.8×10^5	6.0×10^4	4.1×10^4	4.4	<0.3	<0.3	<0.3
July 2/84 Tor.Hum.Soc.	6.7×10^6	6.4×10^6	3.5×10^7	0.2	<0.3	<0.3	0.7
Aug. 12/84 Suburban	1.7×10^6	1.7×10^6	4.5×10^5	3.8	<0.3	<0.3	<0.3
Aug. 19/84 Suburban	6.0×10^4	4.0×10^4	4.0×10^4	1.5	NA	NA	NA
Aug. 6/85 Suburban	1.0×10^3	1.0×10^3	3.0×10^3	1.0	<0.3	<0.3	<0.3
Geometric Means	1.65×10^5	1.2×10^5	1.2×10^5	1.4	<0.3	<0.3	0.4*

All counts recorded per 1 gram of Feces.

* Approximate Value

There is an overall tendency for the levels of indicator bacteria to decrease after stress with fecal coliforms and E. coli decreasing more rapidly than fecal streptococci. This in turn causes a drop in the FC/FS Ratio for most of the samples. The exception to this occurs with muskrat, cat, geese and cow samples which show increased FC/FS ratios after stress. Human FC/FS ratios exhibit the most drastic change, dropping from 27 to 0.1. The least change in ratio occurs in dogs feces which shows a decrease from 13 to 6.7.

Levels of P. aeruginosa increase after stress in the majority of cases. Gulls show the largest increase from 13 to 110 per gram of feces.

3.11 Percent Species of Fecal Coliforms in Human and Non-human Feces from Target Colonies on m-TEC and m-TEC IG (Table 10).

The predominant fecal coliform in the feces of humans and non-humans is E. coli with percent recoveries ranging from 75 to 100%. Humans exhibit the lowest percent recoveries of E. coli (75%) on m-TEC agar but this percentage increases with the use of m-TEC IG (94). Overall, the percent recoveries of E. coli increase with the use of m-TEC IG and there is a tendency towards lower recoveries of Klebsiella and Enterobacter spp. with the medium. Many non-human samples (ie. gulls, geese, pigeons, chickens and cows) exhibited exclusive carriage of E. coli (100%) in their intestinal tract.

TABLE 9: Geometric Mean Levels of Fecal Coliforms, E. coli, Fecal Streptococci, Enterococci and Pseudomonas Aeruginosa and the Fecal Coliform to Fecal Streptococcus Ratios in Stressed Fecal Samples (1984).

SAMPLE SOURCE	FECAL COLIFORMS (M-TEC)	E. COLI (UREASE)	E. COLI (M-TEC IG)	FECAL STREPTOCOCCI (M-ENT)	FECAL STREPTOCOCCI (KF)	ENTEROCOCCI M-ME	F. COLIFORM/ F. STREPTOCOCCUS RATIO M-TEC/M-ENT	P. AERUGINOSA PER 1 GRAM FECES
Humans	$1.0x10^5$	$9.7x10^4$	$1.0x10^5$	$1.1x10^6$	$1.5x10^6$	$1.3x10^6$	0.1	0.73*
Gulls	$1.6x10^7$*	$1.5x10^7$*	$1.2x10^7$*	$3.7x10^6$	$3.95x10^6$	$2.1x10^6$	4.2	110*
Ducks	$2.0x10^6$	$2.0x10^6$	$1.8x10^6$	$2.3x10^6$	$5.1x10^6$	$1.2x10^6$	0.9	1.0*
Geese	$1.9x10^5$	$1.9x10^5$	$9.2x10^4$	$1.9x10^5$	$3.6x10^5$	$1.0x10^5$	1.0	<0.3
Pigeons (Domestic)	$9.7x10^4$*	$1.1x10^4$*	$2.7x10^5$*	$1.1x10^5$	$3.0x10^6$	$1.0x10^6$*	0.9	<0.3
Dogs	$6.8x10^5$	$5.4x10^5$	$6.2x10^5$	$1.0x10^5$	$6.4x10^5$	$1.3x10^6$	6.7	<0.3
Cats	$3.1x10^6$	$3.1x10^6$	$2.8x10^6$	$5.5x10^5$	$8.1x10^5$	$3.5x10^5$	5.6	<0.3
Chickens	$9.0x10^5$	$9.0x10^5$	$2.0x10^5$	$4.2x10^6$	$9.0x10^6$	$5.7x10^6$	0.2	0.7*
Pigs	$9.1x10^5$	$9.1x10^5$	$1.0x10^6$	$5.7x10^5$	$1.5x10^6$	$4.25x10^5$	1.6	0.3*
Cows	$4.4x10^4$	$4.4x10^4$	$3.0x10^4$	$2.6x10^5$	$3.9x10^5$	$3.8x10^5$	0.2	0.4*
Muskrats	$1.3x10^6$	$1.2x10^6$	$1.5x10^6$	$2.6x10^5$	$5.7x10^5$	$1.6x10^5$	5.1	0.6*
Racoons	$8.2x10^5$	$8.1x10^5$	$3.3x10^5$	$2.5x10^7$	$1.4x10^7$	$3.3x10^7$	0.03	5.7*

* Approximate values

TABLE 10: Percent Species of Fecal Coliforms In Humans and Non-Human Feces from Target Colonies on m-TEC (IG) (1984 Data).

ANIMAL	TOTAL M-TEC TARGETS	SPECIES E. COLI	KLEBSIELLA	ENTEROBACTER	CITROBACTER	OTHER	TOTAL M-TEC (IG) TARGETS	SPECIES E. COLI	KLEBSIELLA	ENTEROBACTER	CITROBACTER	OTHER
Gulls	37	37 (100)	-	-	-	-	35	35 (100)	-	-	-	-
Ducks	45	44 (93)	-	-	1 (2.0)	-	45	44 (98)	-	-	1 (2.0)	-
Geese	25	25 (100)	-	-	-	-	30	30 (100)	-	-	-	-
Pigeons	45	45 (100)	-	-	-	-	45	44 (98)	-	-	-	1 (2.0)
Dogs	53	44 (83)	9 (17)	-	-	-	40	35 (87.5)	4 (10)	-	-	1 (2.5)
Cats	44	39 (89)	-	5 (11.0)	-	-	37	35 (95)	-	-	2 (5.0)	-
Pigs	50	49 (98)	-	-	-	1 (2)	45	45 (100)	-	-	-	-
Chickens	39	39 (100)	-	-	-	-	36	33 (92)	-	-	-	3 (8)
Cows	35	35 (100)	-	-	-	-	45	45 (100)	-	-	-	-
Muskrats	34	33 (97)	-	1 (3)	-	-	37	37 (100)	-	-	-	-
Racoons	29	22 (76)	6 (18.1)	-	1 (3.0)	-	26	23 (88.5)	3 (11.5)	-	-	-
Humans	44	33 (75)	8 (21)	3 (6.8)	-	-	36	34 (94)	2 (5.6)	-	-	-

Percentages in Parenthesis
All M-TEC Targets were urease negative

(Strep species Table 13)

3.12 The Percent Species of Fecal Coliforms in Human Feces (Table 11).

Data from the 1984 study and additional work undertaken in 1985 are presented according to the sex of the Host. Again it can be seen that E. coli emerges as the predominant species of fecal coliform bacteria found in humans. However, there does appear to be some differences in percentage recovery of E. coli between the men and women subjects studied. The male hosts tend to carry low percentages of other coliform bacteria (ie. Klebsiella and Citrobacter spp.) while females exhibit 100% carriage of E. coli in their intestinal tracts. The composite sample shows percent recoveries of Enterobacter spp. in addition to the Klebsiella, Citrobacter and E. coli recovered.

3.13 Percent Species of Fecal Streptococci in Human, and Non-human Feces from Target Colonies on m-Enterococcus KF and m-ME Agars Isolated Before Stress (Table 12A & B).

Table 12A presents the percent recovery of the different bacterial species while 12B demonstrates the recoveries from each type of feces in descending order.

Overall, the fecal streptococcus populations found in human and non-human feces are more heterogenious than the fecal coliform populations. A considerable amount of variation exists between the sources tested and no two sources are completely alike in their representative populations.

TABLE 11: The Percent Species of Fecal Coliforms in Human Feces 1984 - 1985.

SUBJECT	TOTAL ISOLATES	TOTAL FECAL COLIFORMS	E. COLI	KLEBSIELLA SPP.	ENTEROBACTER SPP.	CITROBACTER SPP.	NON-FECAL COLIFORMS
Males 20-30 yrs	60	60 (100)	49 (93.4)	10 (6.0)	-	1 (0.6)	-
Females 20-30 yrs	60	60 (100)	60 (100)	-	-	-	-
Composite Male & Female 20-30 yrs	64	64 (100)	32 (50.0)	18 (28.1)	5 (7.8)	9 (14.1)	- -

Percentages in Parenthises

TABLE 12A: Percent Species of Fecal Streptococci in Human and Non-Human Feces From Target Colonies on m-Enterococcus m-ME and KF Agar Isolated Before Stress (1984).

SOURCE	TOTAL ISOLATES	S. FAECALIS VAR. LIQ	S. FAECALIS VAR. FAECALIS	S. FAECALIS VAR. ZYM	S. FAECIUM VAR. FAECIUM	S. FAECIUM VAR. CASSELIFLAVUS	S. DURANS	S. BOVIS	S. BOVIS VAR.	S. EQUINUS	S. AVIUM	AEROCOCCUS	F.S.*	NON + F.S.	STAPHYLOCOCCI
Humans	188	38 (20)	6 (3.2)	2 (1.1)	79 (42)	-	42 (22.3)	5 (2.65)	1 (0.5)	-	-	-	3 (1.6)	9 (4.8)	3 (1.6)
Gulls	227	89 (39.2)	9 (4.0)	17 (7.5)	38 (16.7)	16 (7.0)	8 (3.5)	33 (14.5)	1 (0.4)	-	1 (0.4)	-	-	10 (4.4)	5 (2.2)
Ducks	194	56 (28.9)	19 (9.8)	8 (4.1)	57 (29.4)	19 (9.8)	18 (9.3)	-	-	-	-	1 (0.5)	2 (1.0)	2 (1.0)	12 (6.2)
Geese	255	63 (24.7)	8 (3.1)	2 (0.8)	35 (13.7)	100 (39.2)	43 (16.9)	-	-	-	-	-	-	-	4 (1.6)
Pigeons (Domestic)	126	7 (5.5)	1 (0.8)	-	10 (7.9)	15 (11.9)	21 (16.7)	-	-	-	1 (0.8)	68 (54.0)	2 (0.15)	-	1 (0.8)
Pigeons° (Wild)	151	68 (45.0)	5 (3.3)	-	6 (4.0)	5 (3.3)	55 (36.4)	-	1 (0.7)	-	-	-	-	1 (0.7)	10 (6.6)
Dogs	257	18 (7.0)	2 (0.8)	6 (2.3)	59 (22.9)	4 (1.6)	37 (14.4)	61 (23.7)	50 (19.4)	-	11 (4.3)	2 (0.8)	4 (1.6)	1 (0.4)	2 (0.8)
Cats	204	5 (2.45)	7 (3.4)	-	6 (2.9)	1 (0.5)	59 (29.0)	36 (17.6)	-	-	-	-	2 (1.0)	79 (38.7)	9 (4.4)
Chickens	186	36 (19.3)	1 (0.5)	2 (1.1)	54 (29.0)	18 (9.7)	12 (6.45)	3 (1.6)	-	-	8 (4.3)	16 (8.6)	2 (1.1)	13 (7.0)	21 (11.3)
Pigs	138	5 (3.6)	1 0.7	-	27 (19.6)	1 (0.7)	42 (30.4)	3 (2.2)	-	-	1 (0.7)	21 (15.2)	-	12 (8.7)	15 (10.9)
Cows	201	2 (1.0)	-	13 (6.5)	7 (3.5)	-	10 (5.0)	71 (35.3)	3 (1.5)	-	-	51 (25.4)	3 (1.5)	14 (7.0)	27 (13.4)
Muskrats	145	67 (46.2)	11 (7.6)	4 (2.7)	-	37 (25.5)	-	7 (4.8)	-	-	2 (1.4)	12 (8.3)	2 (1.4)	2 (1.4)	1 (0.7)
Racoons	125	26 (21.0)	4 (3.2)	1 (0.8)	45 (36.0)	6 (4.8)	3 (2.4)	20 (1.6)	7 (5.6)	-	-	-	3 (2.4)	10 (8.0)	-
Horses°	87	3 (3.4)	-	1 (1.1)	16 (18.4)	10 (11.5)	35 (40.2)	-	-	20 (23.0)	-	-	-	2 (2.3)	-
Turkeys°	53	48 (90.6)	-	-	-	-	1 (1.9)	-	-	-	-	-	2 (3.8)	-	2 (3.8)

* The F.S. grouping is comprised of fecal streptococci which were non-indentifiable by biochemical methods but could be identified serologically by their group D antigen.

+ The Non F.S. grouping is comprised of Gram (+), Catalase (-), Non-Group D.

° 1985 Data.

Percentages in Parenthesis

Legend for Table 12B and 15B

GENUS & SPECIES	CODE
S. Faecalis var. liquifaciens	SFL
S. faecalis faecalis	SFF
S. faecalis Zymnogenes	SFZ
S. faecium	SFM
S. faecuim var. casseliflavus	SFmC
S. durans	SD
S. bovis	SB
S. bovis (variant)	S Bv
S. avium	SA
Aerococcus spp.	AER
Non-Fecal Streptococci	NFS
Staphycococcus	STAPH

(unidentified FS not used).

Notations:

>> Difference ≥ 10%

> Difference ≥ 5% < 10%

≥ Difference < 5% > 1%

= Difference < 1%

* (+ one of the above notations) % recovery drops below 10%

TABLE 12B: Relative Recovery from Feces of Fecal Streptococci and Other Bacteria Isolated as Target Colonies on the Streptococcus Recovery Media Before Stress.

SOURCE	ORDER OF RECOVERY FROM FECES BY DECREASING PERCENT POPULATION	
Humans	SEm >> SD > SEL *>> NES ≥ SEF = SB ≥ SIAEH = SEZ = SBv >	(0) SEmC, SA, AER & SE
Gulls	SEL >> SEm ≥ SB *>> SEZ = SEmC ≥ NES = SEF = SD ≥ SIAEH ≥ SA = SBv >	(0) AER & SE
Ducks	SEm = SEL *>> SEF = SEmC = SD ≥ SIAEH ≥ SEZ ≥ NES = AER >	(0) SB, SBv, SA & SE
Geese	SEmC >> SEL > SD ≥ SEm *>> SEF ≥ SIAEH ≥ SEZ >	(0) SB, SBv, SA, AER, NES & SE
Pigeons (Domestic)	AER >> SD > SEmC *>> SEm ≥ SEL > SEF = SA = SIAEH >	(0) SEZ, SB, SBv, NES & SE
Pigeons (Wild)	SEL > SD *>> SIAEH ≥ SEm = SEF = SEmC ≥ SBv = NES >	(0) SEZ, SB, SA, AER & SE
Dogs	SB ≥ SEm ≥ SBv > SD *>> SEL ≥ SA ≥ SEZ ≥ SEmC = SEF = AER = SIAEH = NES >	(0) SE
Cats	NES ≥ SD >> SB *>> SIAEH ≥ SEF = SEm = SEL ≥ SEmC >	(0) SEZ, SBv, SA, AER & SE
Chickens	SEm >> SEL > SIAEH ≥ SEmC *≥ AER ≥ NES = SD ≥ SA ≥ SB = SEZ = SEF >	(0) SBv & SE
Pigs	SD >> SEm > AER > SIAEH*≥ NES = SEF ≥ SEL ≥ SB ≥ SEmC = SA >	(0) SEZ, SBv & SE
Cows	SB >> AER >> SIAEH*> NES = SEZ ≥ SD ≥ SEm ≥ SBv = SEL >	(0) SEF, SEmC, SA & SE
Muskrats	SEL >> SEmC *>> AER = SEF ≥ SB ≥ SEZ ≥ SA = NES = SIAEH >	(0) SEm, SD, SBv & SE
Racoons	SEm >> SEL *>> NES ≥ SBv ≥ SEmC ≥ SEF ≥ SD = SB = SEZ >	(0) SA, AER, SIAEH & SE
Horses	SD >> SE > SEm > SEmC *>> SEL ≥ NES ≥ SEZ >	(0) SEF, SB, SBv, SA, AER & SIAEH
Turkeys	SEL *>> SIAEH > SD >	(0) SEF, SEZ, SEm SEmC, SB, SBv, SA, AER, NES & SE

Human feces exhibited the highest percentage population of S. faecium (42%). However, high percent recoveries of this organism were also present in raccoons (36%), ducks (29.4%), chickens (29%] and dogs (22%). Both S. faecalis var. liquefaciens and S. durans were present in human feces to an approximately equal degree and at one half of the percent level of S. faecium present. Raccoons and geese also had similar percent recoveries of S. faecalis var. liqufaciens (21% & 24.7% respectively) but exhibited lower precent levels of S. durans (2.4% & 16.9% respectively). The highest percent population of S. faecalis var. liquefaciens occurred in muskrats (46.2%) followed by wild pigeons (45%), gulls (39.2%) and ducks (28.9%). The highest percent recovery of S. durans occurred in horses (40%), wild pigeons (36.4%), pigs (30.4%) and cats (29%).

Species of streptococci rarely or never found in human feces but recovered more often from non-human were: S. faecium var. casseliflavus (geese 39.2%, muskrats 25.5%, domestic pigeons 11.9%), S. bovis and S. bovis variant (cows 35.3%, dogs 23.7% and 19.4%, cats 17.6% and <0.5%, gulls 14.5%); Aerococcus (domestic pigeons 54%, cows 25.4%, pigs 15.2%, chickens 8.6%) and non-fecal Streptococci (cats 38.7%, pigs 8.7%, raccoons 8% and cows 7%) S. equinus was only isolated from horse feces.

3.14 Percentage of S. faecalis var. from Human and Non-Human Feces Giving Proteinization Reactions in Litmus Milk (Table 13).

A major difference occurring between the varieties of S. faecalis isolated from human and non-human feces is that the

TABLE 13: Percentage of S. Faecalis var. from Human and Non-Human Feces Giving Proteinization Reactions in Litmus Milk.

SOURCE	TOTAL NUMBER ISOLATES	ACID CURD	ALKALINE (RENNET) CURD	PEPTONIZATION (DIGESTION)	REDUCTION	NO REACTION
Humans	63	10 (15.9)	51 (81.0)	-	2 (3.2)	-
Gulls	32	-	1 (3.1)	31 (97.0)	-	-
Ducks	15	1 (6.7)	-	2 (13.3)	-	12 (80.0)
Geese	31	2 (6.4)	26 (83.9)	2 (6.4)	-	1 (3.2)
Pigeons (Domestic)	67	-	51 (76.1)	12 (17.9)	-	4 (6.0)
Pigeons (Wild)	71	-	53 (74.6)	14 (19.7)	2 (2.8)	2 (2.8)
Dogs	24	4 (16.7)	4 (16.7)	15 (62.5)	1 (4.2)	-
Cats	21	-	2 (9.5)	16 (76.2)	-	3 (14.3)
Chickens	10	2 (20)	1 (10)	7 (70.0)	-	-
Pigs	3	-	-	1 (33.3)	2 (66.7)	-
Cows	25	1 (4.0)	5 (20.0)	16 (64.0)	3 (12)	-
Muskrats	29	5 (17.2)	2 (6.9)	18 (62.1)	4 (13.8)	-

Incubation Period for Litmus Milk test was 7 days - tubes read daily
Percentages in Parentheses

human biotypes do not peptonize milk. However, it can be seen that not all of the animal biotypes produced peptonization reactions either. Some samples (ie. geese, domestic & wild pigeons) carried mainly rennet curd producing varieties of S. faecalis as did humans. However, all of the non-human samples did carry some percentage of the proteinizing variety.

3.15 The Percent Species of Fecal Streptococci and Enterococci in Human Feces (Table 14).

Combined results from 1984 and 1985 studies of the fecal streptococcus populations in 13 humans demonstrate the variability in recovery that can occur within the human species (Note: This data includes results not incorporated into Table 12). Differences between male and female subjects are evident. In females, S. durans (35%) seems to be the more predominant species isolated, with S. faecium (28.75%) and S. faecalis (18.75%) also present to a substantial degree. In males however, the recovery of both S. faecalis var liquefaciens and S. durans is quite low (0.625 and 3.1% respectively) and S. faecium (88.75%) is present almost to the exclusion of everything else. It is interesting to note that the composite male and female sample made up of different subjects from those used in the separate male and female groups, does not bear any resemblance to either of the individual samples. In the composite sample, S. faecium is recovered at a very low concentration despite the fact that this organism makes up a significant portion of the fecal strepcococcus population in both the separately tested male

TABLE 14: The Percent Species of Fecal Streptococci and Enterococci in Human Feces

SUBJECT	TOTAL ISOLATES	S. FAECALIS VAR.			S. FAECIUM VAR.		S. DURANS	S. BOVIS	S. BOVIS VAR.	S. AVIUM	F.S.*	NON. F.S.	STAPH.
		FAECALIS	LIQUE-FACIENS	ZYMO-GENES	FAECIUM	CASSE-LIFLAVUS							
Males 20-50 yrs	160	2 (1.25)	1 (0.625)	1 (0.625	142 (88.75)	-	5 (3.1)	5 (3.1)	1 (0.625)	-	1 (0.625)	2 (1.25)	-
Females 20-30 yrs	80	1 (1.25)	15 (18.75)	-	23 (28.75)	-	28 (35.0)	-	-	-	1 (1.25)	7 (8.75)	5 (6.25)
Composite Male & Female 20-30 yrs	97	8 (8.2)	53 (54.6)	1 (1.03)	9 (9.3)	-	24 (24.7)	-	-	-	1 (1.03)	1 (1.03)	-

() Percentages in Parenthises

and female subjects. Also, S. faecalis var liquefaciens (54.6%) is present at a much higher concentration than was displayed in the separate samples.

Another interesting fact, is the low recovery of S. bovis (3.1) and S. bovis var (0.625%) in the male group. These organisms were isolated from the feces of a 50 to 55 year old resident in the Bolton area. Even with a low recovery of S. bovis and of non-fecal streptococci, the majority of the fecal streptococcus population in humans fell within the Enterococci group.

3.16 Percent Species of Fecal Streptococci in Human and Non-human Feces from Target Colonies on m-Enterococcus KF and m-ME Agars Isolated after Stress (Tables 15A & B).

Table 15A shows the proportional recovery of the different bacterial species isolated while 15B presents the recoveries from each type of feces in decreasing order.

Recoveries of fecal streptococci after the 24 hour stress period demonstrates again considerable variation between the sources tested. However, a number of changes as a result of stress, have occurred. In all cases the proportional distribution of species and variants have changed sometimes drastically. In humans the S. faecium percent population is now 58% which is an increase over the pre-stress recoveries. In fact only two of the fecal types (domestic pigeons & pigs) exhibited lower percent recoveries of S. faecium after stress. Proportional recoveries of S. faecalis var.

TABLE 15A: Percent Species of Fecal Streptococci in Humans and Non-Human Feces from Target Colonies on m-Enterococcus m-ME and KF Agar Isolated After Stress (1984)

SOURCE	TOTAL ISOLATES	S. FAECALIS VAR. LIQ	FAECALIS	ZYM	S. FAECIUM VAR. FAECIUM	CASSELIFLAVUS	S. DURANS	S. BOVIS	S. BOVIS VAR.	S. EQUINUS	S. AVIUM	AEROCOCCUS	F.S.*	NON + F.S.	STAPHYLOCOCCI
Humans	130	37 (28)	5 (4)	-	75 (58)	-	11 (8)	-	-	-	-	-	-	-	2 (2)
Gulls	83	11 (13)	1 (1)	6 (7)	40 (48)	5 (6)	1 (1)	10 (12)	2 (2)	-	-	-	2 (2)	1 (1)	4 (5)
Ducks	95	14 (15)	12 (13)	1 (1)	37 (39)	3 (3)	17 (18)	-	-	-	1 (1)	7 (7)	-	-	3 (3)
Geese	155	55 (35)	3 (2)	-	28 (18)	38 (15)	27 (7)	-	-	-	-	-	-	-	5 (3.5)
Pigeons (Domestic)	47	-	-	-	3 (6)	15 (30)	17 (34)	-	-	-	-	11 (22)	-	1 (2)	-
Dogs	142	5 (3.5)	6 (4)	20 (14)	54 (38)	-	19 (13)	21 (15)	9 (6)	-	2 (1.5)	-	-	3 (2)	3 (2)
Cats	141	12 (9)	10 (7)	11 (8)	5 (3.5)	-	22 (16)	15 (11)	1 (1)	-	-	-	10 (7)	37 (26)	5 (3.5)
Chickens	64	-	-	2 (3)	46 (72)	-	4 (6)	-	-	-	-	1 (1)	-	3 (5)	8 (13)
Pigs	136	10 (7)	1 (1)	-	7 (5)	2 (2)	41 (30)	-	-	-	-	44 (32)	5 (4)	9 (7)	13 (10)
Cows	138	-	-	30 (22)	11 (8)	2 (1.5)	23 (17)	36 (26)	-	-	-	19 (14)	2 (1.5)	-	9 (7)
Muskrats	99	13 (13)	34 (33)	8 (8)	17 (17)	17 (17)	1 (1)	-	-	-	-	1 (1)	-	6 (6)	2 (2)
Raccons	66	5 (7.6)	2 (3.0)	2 (3.5)	27 (40.9)	-	7 (10.6)	2 (3.0)	-	1 (1.5)	1 (1.5)	-	5 (7.6)	13 (19.7)	1 (1.5)

Percentages in Parenthesis ( )

TABLE 15B: Relative Recovery from Feces of Fecal Streptococci and Other Bacteria Isolated or Target Colonies on the Streptococcus Recovery Media After Stress.

SOURCE	ORDER OF RECOVERY FROM FECES BY DECREASING PERCENT POPULATION	
Humans	SEm >> SEL *>> SD ≥ SEF ≥ SIAEH >	(0) SEZ, SEmC, SB, SBv, SE, SA, AER & NES
Gulls	SEm >> SEL = SB *> SEZ ≥ SEmC = SIAEH ≥ SBv = SEF = SD = NES >	(0) SE, SA & AER
Ducks	SEm >> SD ≥ SEL ≥ SEF *>> AER ≥ SEmC = SIAEH ≥ SEZ = SA >	(0) SB, SBv, SE & NES
Geese	SEL > NES > SEm ≥ SEmC *> SD ≥ SIAEH ≥ SEF >	(0) SB, SBv, SE, SA & AER
Pigeons (Domestic)	SD ≥ SEmC > AER *>> SEm ≥ NES >	(0) SEL, SEF, SEZ, SB SBv, SE, SA & SIAEH
Dogs	SEm >> SB = SEZ = SD *> SBv ≥ SEF = SEL ≥ NES = SIAEH = SA >	(0) SEmC, SE & AER
Cats	NES >> SD > SB *≥ SEL = SEZ = SEF ≥ SEm = SIAEH ≥ SBv	(0) SEmC, SE, SA & AER
Chickens	SEm >> SIAEH*> SD = NESC ≥ SEZ ≥ AER >	(0) SEL, SEF, SEmC, SB, SBv, SE & SA
Pigs	AER ≥ SD >> SIAEH*≥ SEL = NES ≥ SEm ≥ SEmC = SEF >	(0) SEZ, SB, SBv, SE & SA
Cows	SB ≥ SEZ > SD ≥ AER *> SEm = SIAEH > SEmC >	(0) SEL, SEF, SBv, SE, SA & NES
Muskrats	SEF >> SEm = SEmC ≥ SEL *> SEZ ≥ NES > SIAEH = SD = AER >	(0) SB, SBv, SE & SA
Raccons	SEm >> NES >> SD *≥ SEL > SEF = SEZ = SB ≥ SE = SA = SIAEH >	(0) SEmC, SBv & AER

liquefaciens increase slightly in humans from 20 to 28% while recoveries of S. durans drop from 22.3 to 8%. The increase in S. faecalis var. liquefaciens displayed in humans was not seen in most non-human feces where decreases in this organism occurred in 8 out of the 12 samples. A decrease in S. durans among non-humans occurred in 7 out of the 12 samples. Recoveries of S. bovis and S. avium decreased in all of the fecal samples after stress as did proportional levels of Aerococcus.

3.17 Salmonella Serotypes Isolated from Animal Feces (Table 16A & 16B).

A wide variety of salmonella serotypes seem to be present in non-human feces particularly in wild fowl. There is a significant presence of Salmonella typhimurium in the feces of gulls, ducks and geese, as well as S. agona (gulls & ducks) and S. californnia (ducks). The recovery of salmonella from raccoons occurred only in one of the two samples analysed. This particular subject came from the same farm that some of the chicken samples were obtained from. However, the Salmonella serotypes isolated from the raccoon are totally different to those isolated from the chicken samples.

3.18 Percent Species of Campylobacter in Animal Feces (Tables 17A & 17B).

Analysis of non-human samples during 1984 and 1985 reveal a substantial carriage rate of Campylobacter spp. among non-humans. Only 4 out of the 14 animal samples tested did not

ABLE 16A: Salmonella Serotypes Isolated from Animal Feces

SOURCE	S. TYPHIMURIUM	S. AGONA	S. BRANDENBURG	S. SENF+ENBERG	S. ORANIENBURG	S. HEIDELBERG	S. CALIFORNIA	S. BONARIENSIS	S. HADAR	S. INFANTIS
Gulls	4	1	-	-	1	1	-	-	1	-
Ducks	3	2	-	-	-	-	8	1	-	-
Geese	3	-	-	-	-	-	-	-	-	-
Chicken	-	-	1	7	-	-	-	-	-	-
Racoon	-	-	-	-	-	-	-	-	-	8

TABLE 16B: Antibiotic Resistance Patterns in Salmonella Serotypes Isolated from Animal Feces

STRAIN	SEROTYPE	SOURCE		PLASMIDS	RESISTANT TO: Ap 16	Ap 8	Ti 100	Ti 50	Cx 10	Cm 8	Cm 4	Tm 8	Tm 4	Tc 4	Pb 10	Nf 30	Na 12	Cm 16	Sx 10	Ca 50	Ce	Ka 30	Cs 10
RC-1	S. infantis	Raccoon	No													+			+				
RC-2	S. infantis	Raccoon	No													+			+				
RC-3	S. infantis	Raccoon	No													+			+				
RC-4	S. infantis	Raccoon	No													+			+				
RC-6	S. infantis	Raccoon	No													+			+				
RC-7	S. infantis	Raccoon	No													+			+				
RC-8	S. infantis	Raccoon	No													+			+				
Du-6	S. california	Duck	No																				
Du-7	S. california	Duck	No													+			+				
Du-8	S. california	Duck	No													+			+				
Du-9	S. california	Duck	No													+			+				
Du-10	S. california	Duck	No													+			+				
Du-12	S. california	Duck	No													+			+				
Du-13	S. california	Duck	No													+			+				
Du-14	S. california	Duck	No													+			+				
Ch-2	S. saftenberg	Chicken	Yes																				
Ch-3	S. saftenberg	Chicken	Yes																+				
Ch-4	S. saftenberg	Chicken	Yes	66,56,10.9,10.15,															+				
Ch-5	S. saftenberg	Chicken	Yes	10,8,5.8,5.0,															+				
Ch-6	S. saftenberg	Chicken	Yes	4.4,3.6,3.3,															+				
Ch-7	S. saftenberg	Chicken	Yes	2.6,1.9,1.4															+				
Ch-8	S. saftenberg	Chicken	Yes																+				
Ch-1	S. branderburg	Chicken	Yes	6,2.65												+			+				

TABLE 16B: Antibiotic Resistance Patterns in Salmonella Serotypes Isolated from Animal Feces (Cont'd)

STRAIN	SEROTYPE	SOURCE		PLASMIDS	RESISTANT TO: Ap 16	Ap 8	Ti 100	Ti 50	Cx 10	Gn 8	Gn 4	Tm 8	Tm 4	Tc 4	Bo 10	Nf 30	Na 12	Cn 16	Sx 10	Ca 50	Ce	Ka 30	Cs 10
Du-1	S. typhimurium	Duck	Yes	11.2,11,7												+			+				
Du-2	S. typhimurium	Duck	Yes	11.2,11,7												+			+				
Ge-1	S. typhimurium	Goose	Yes	8.6,7.0															+				
Ge-2	S. typhimurium	Goose	Yes	66,10.8,5.2,4.5															+				
Gu-6	S. typhimurium	Gull	Yes	4.6,4,2												+			+				
Gu-7	S. typhimurium	Gull	Nb													+			+				
Du-3	S. agona	Duck	Yes	10.15,5												+			+				
Du-4	S. agona	Duck	Yes	3.8												+			+				
Gu-8	S. agona	Gull	Yes	62,1.1												+			+				
Du-11	S. bonariensis	Duck	Nb													+			+				
Gu-4	S. hadar	Gull	Nb													+			+				
Gu-5	S. oranienburg	Gull	Nb													+			+				
Gu-1	S. heidelberg	Gull	Yes	11.4,11,10.8,10.3, 5.75,4.4,1.75	+	+	+	+								+			+	+	+		
Gu-1-Sal	S. typhimurium	Transconjugant		62,11.4,5.75,2,1.25	+	+	+	+								+	+		+	+	+		
Sal	S. typhimurium	Recep.		62,2,1.25													+		+				

TABLE 17A: Percent Species of Campylobacter in Animal Feces Sampled in 1984 and 1985

SOURCE	TOTAL ISOLATES	C. JEJUNII	C. COLI	C. LARIDIS	NOT + IDENTIFIABLE
Gulls	61	32 (52.5)	-	23 (37.7)	6 (9.8)
Ducks	10	2 (20)	-	7 (70)	1 (10)
Geese	18	14 (77.8)	-	2 (11.1)	2 (11.1)
Pigeons (Wild)	29	20 (69)	-	-	9 (31)
Chickens	10	10 (100)	-	-	-
Turkeys	7	7 (100)	-	-	-
Pigs	4	-	4 (100)	-	-
Cows	3	3 (100)			
Dogs	10	8 (80)	-	-	2 (20)
Cats	4	4 (100)			

+ not identifiable by biochemical means

TABLE 17B: Percent Occurrence of Campylobacter spp. in Gull (Laridis spp.) Feces Sampled from Humber West Park, 1984.

NO. OF INDIVIDUAL FECAL SAMPLES	NO POSITIVE FOR CAMPYLOBACTER SPP.	NO. POSITIVE FOR C. JEJUNII	NO POSITIVE FOR C. LARIDIS
40	13 (32.5)	2 (5)	11 (27.5)

harbour this organism. There is a significant percentage of the pathogen Campylobacter jejunii in gull, geese, wild pigeons, chicken, turkey and dog feces. Pigs were shown to be the only source of E. coli in the study and waterfowl (ie. gulls, ducks and geese) the only carriers of C. laridis. Results from a separate 1984 study are presented in Table 17B and demonstrate the percent occurrence of Campylobacter spp. among gulls. A total of 32.5% of the 40 gulls analysed carried Camplylobacter spp. in their feces. Of these samples 5% were positive for C. jejunii. These finding when compared to those in Table 17A show again the variability that can occur within a species population.

4.0 DISCUSSION

4.1 Concentrations of Fecal Indicator Bacteria and Enteric Pathogens in Human and Non-Human Feces (Table 1).

4.1.1 Fecal Coliforms and E. coli:

A bacterial species, to be considered a good indicator of fecal, pollution, must adhere to a number of criteria. These criteria include; (1) high densities in feces far exceeding pathogen levels; (2) no other environmental source except the feces of warm blooded animals; (3) a positive correlation between the indicator and fecal contamination and between the indicator and waters contaminated with feces and (4) that once discharged into the environment, the persistence and regrowth (survival) characteristics of the indicator parallel that of the most persistent pathogens (Geldreich, 1978).

Previous studies have shown that E. coli possesses most of these traits and it has been suggested as the fecal pollution indicator of choice (Palmer, Lock and Gowda, 1984; Jones and White, 1983; Dufour, Strickland, 1979; Dufour, 1977; Barrow 1977; Mitchell and Starzyk, 1975).

The purpose of this study was not to re-establish E. coli as a good fecal parameter but to assess the performance of current methods to ensure the reliability

of the data provided in the interpretation of water quality studies.

The concentrations of fecal coliforms (FC) and E. coli (EC) in the feces analyzed (Table 1) are in agreement with levels reported in previous studies (Jones and White, 1983; Wheater, Mara and Oragui, 1978; Geldreich, 1976; Geldreich and Kenner, 1969). The densities of both FC and EC are high with their respective levels showing good correlation with each other. It can also be seen (Table 1) that there is good agreement between the two E. coli procedures, ie. m-TEC + Urease and m-TEC IG which tends to give a higher level of confidence to the results obtained. The fact that there is some disagreement between the two media is probably due to such factors as the types of fecal coliforms other than E. coli carried by the host eg. urease negative FC, and the different levels of performance of the two procedures; for instance the m-TEC IG medium has a higher specificity for the recovery of EC (Dufour 1979).

The levels of E. coli obtained using either recovery method demonstrated a number of non-human feces with higher EC levels than humans. This may in part be due to the highly polluted environment in which they live. The high levels of E. coli found in Toronto waterfowl (ie. Gulls, Ducks and Geese) may be attributed to association with water polluted by human feces (Mead, 1972), whereas, birds having less contact with the

human environment have lower levels of E. coli and other indicator organisms (Mead, 1965; Mundt, 1963; Mushin and Ashburner, 1962). Diet may effect the concentration of different intestinal bacteria (Mead, 1972) and this may account for higher levels of E. coli in pigeons and raccoons that can scavenge human garbage and domesticated pets which frequently consume human food. In a study of the bacterial flora of cows, diet was shown to have an effect on bacterial levels. Cattle kept outdoors and grazed in a natural environment were found to exhibit higher levels of E. coli than cattle kept completely indoors. (Mara and Oragui, 1978). Regardless of the species or diet it would appear that the levels of E. coli are high in all feces examined during this study.

The Metropolitan Toronto area contains large populations of humans and many types of animals and birds both domesticated and wild. It is, therefore, easy to see that a major potential for fecal pollution and excedence of water quality standards exists and in fact frequently occurs. Table 18 lists the daily fecal output of humans, dogs, cows gulls, ducks and pigeons and demonstrates the potential for fecal contamination (>100 FC/100 ml) if only 10% of their fecal material is dissolved in surface waters. These calculations show that a considerable volume of water could potentially be contaminated by one individual in one day. When one also considers the population dynamics of the various species ie. 75,000 nesting pairs of gulls on the Leslie

St. Spit [1] and 20,755 licensed dogs in the city of Toronto) [2] the potential for pollution greatly increases.

[1] Estimated 1984 population levels, provided by Environment Canada

[2] Toronto Humane Society

TABLE 18: Contamination of Surface Waters by 10% of the Total Fecal Output of Humans and Non-Humans

SAMPLE	AVERAGE DAILY* FECAL OUTPUT (GROSS) (WET WEIGHT)	CONCENTRATION OF FECAL COLIFORMS PER FECAL OUTPUT	10% OF DAILY FECAL OUTPUT (GROSS) (WET WEIGHT)	CONCENTRATION OF F. COLIFORMS PER 10% FECAL OUTPUT	VOLUME OF WATER (LITERS) THAT COULD BE POTENTIALLY CONTAMINATED (100 FC/100 ML) BY 10% DAILY FECAL OUTPUT
Humans	150	3.9×10^9	15	3.9×10^8	390,000
Gulls	16.5	3.05×10^9	1.65	3.05×10^8	305,000
Ducks	336	5.0×10^{10}	33.6	5.0×10^9	5,000,000
Dogs	140 (Large size dog)	3.2×10^9	14.0	3.2×10^8	320,000
Cows	23,600	1.2×10^{10}	2,360	1.2×10^9	1,200,000
Pigeons (Wild)	37.5 (Average)	1.2×10^{10}	3.75	1.2×10^9	1,200,000

(* Geldrich, 1977)
(Pitt, 1982)
(Gould & Fletcher, 1978)
(Jones and White, 1983)
(Howe, 1969)

The number of potential input sites for fecal material from human and non-human sources is extensive. There are 624 storm and combined sewer outfalls on the Humber River and tributaries within the Metropolitan Toronto boundaries (Gartner and Lee, 1983) and 46 along the Toronto waterfront (A. Marich, personal communication). Human, dog and other domestic pet waste can be washed into surface waters during wet weather conditions from combined sewer overflows, storm sewers and direct run-off. It has been suggested that dog and other domestic pet wastes are an important source of bacterial contamination in storm water run-off and that these animals should be kept from defecating on streets and near the banks of receiving waters (Schillinger and Stuart, 1978; Marron and Sems, 1974). Human feces may also be carried in storm sewer lines during both wet and dry weather conditions as a result of illegal connections and cross connections with sanitary sewers. The extent these problems is unknown but a recent study of 239 active dry weather outfalls along the Humber River and tributaries reported 84 which had unacceptably high levels of fecal indicator bacteria which could be the result of sanitary waste being present (Gartner and Lee, 1983).

Birds have ready access to surface waters. Wild pigeons living on bridges contribute greatly to water quality deterioration by defecating directly into the water (Palmer, 1983). Waterfowl also defecate directly into surface waters or along the shoreline and where

their populations are substantial, water quality has declined (Oplinger, 1977; Fennell, James and Morris, 1974). A number of areas along the Humber River (ie. James Gardens) support waterfowl populations which roost on small islands or sediment beds in the middle of the river. There are large areas of livestock access to the water in the Upper Humber and Don Rivers. In the Upper Humber and its tributaries, there are 27 access sites ranging in length from 0.01 to 6.75 km (Hindley, Hubbard and Maude, 1985). The impact of fecal material discharge into surface waters by livestock is in general poorly documented (McIlroy et. al., 1975). Cattle grazing near water channels have been shown to have a major impact on the bacterial densities (Kundle, 1970) and water quality has declined in areas of livestock access (Thelin and Gifford, 1983; Robbins, Howells and Kriz, 1972).

4.1.2 Fecal Streptococci and FC/FS Ratios.

The concentrations of fecal streptococci (FS) recovered from feces (Table 1) also parallel results of previous studies and are high in all samples tested. Concentrations of enterococci were also high and identifications demonstrated that except for the case of pigs (Table 1B) they constituted a majority of the FS population. Their high densities is a positive factor in selecting enterococci as a fecal indicator. Previous studies have indicated that enterococci exhibit survival patterns more closely related to viral pathogens

than fecal coliforms do and have indicated that enterococci in conjunction with E. coli would be the indicators of choice for protection of bathers (Palmer et. al., 1984; Dufour, 1982).

The tendency towards higher levels of fecal streptococci on KF agar is due to its being a less restrictive medium than m-Enterococcus agar (Geldreich, 1976). Lower concentrations reported on m-ME are due to its greater specificity for enterococci (Dufour, 1980). In certain non-human cases, recoveries on m-ME were higher and/or recoveries on KF were lower than on m-Enterococcus agar. This may be due to the types of streptococci carried by the host eg. recovery of S. bovis is greater on m-ME than on m-Enterococcus agar (Seyfried, Harris and Young, 1986).

The fecal coliform to fecal streptococci (FC/FS) ratios obtained are in disagreement with those of previous studies (Geldreich, 1972 and 1976) and although human feces had the highest FC/FS ratio (27), many non-human samples also displayed ratios well above 4. Other workers have reported discrepancies in FC/FS ratios obtained from feces. Some attribute the differences to choice of media and the methods used (Palmer, 1984; Kenner, 1972; Diebel, 1964). Since the recovery methods used in this study are different from those used originally to develop FC/FS ratios, it is possible that the ratios have been affected. The comparison of FC/FS ratios of human and non-human feces computed from fecal

coliform, E. coli, fecal streptococci and enterococci levels recovered on the various media (Table 1A) shows that the use of E. coli data (m-TEC plus urease and m-TEC IG) in place of fecal coliform levels (m-TEC) affects a change in the ratios which is more evident with E. coli urease than with E. coli IG. Changing the streptococcus recovery media from m-Enterococcus to either KF or m-ME creates an even greater shift in ratios. An increase in the spread between human and non-human FC/FS ratios occurs when m-TEC plus urease data is used in combination with KF or m-ME, for instance, there are fewer non-human feces with FC/FS ratios above 10. It is obvious that changing the media can have a dramatic effect on the FC/FS ratios and that the choice of media is important. The best combination for ratio determination may be to use E. coli (m-TEC plus urease) densities with fecal streptococci levels determined on KF by the original spread plate method. The KF medium, however, has a poorer specificity for enterococci than either m-Ent or m-ME thus recovering bacteria unrelated to fecal pollution. It may be better to investigate the use of m-ME for determining FC/FS ratios since it appeared to provide somewhat better differentiation of enterococci than m-Ent and provides a better water quality assessment method.

Many studies suggest factors such as diet (Hussong et. al., 1978; Drasar and Hill, 1974; Wilkens and Long, 1971; Ducluzean et. al., 1965) and association with a human fecal polluted environment (Mead, 1972) as having

a major influence on fecal coliform to fecal streptococci ratios. Geographic location may be the most important factor in determining the FC/FS ratios in feces since most of the above factors (ie. diet, stress, pollution) are directly related to the area of habitation of the host. Fecal samples obtained from birds and wild animals living in an unpopulated, pristine area could yield different FC/FS ratios to those taken from the same types of animals living in a highly urbanized and polluted environment like that of the Metro Toronto area. It is interesting to note that in this study many of the non-human subjects exhibiting high FC/FS ratios (similar to humans) are those which have a close association with humans (dogs) or with human polluted environments (gulls, ducks, wild pigeons and muskrats). The elevated ratios of these particular subjects may be due, at least in part, to the fact that they reside in the Metro Toronto area.

Variation of the fecal coliform to fecal streptococcus ratios within the same species of host and between different species living in the same location and sharing the same food source has also been documented. In a study of gull feces by Gould and Fletcher (1978), a wide variety of ratios were reported from gull samples obtained at the same garbage dump. Different ratios ranging from 87 to 575 were reported among the different species of gulls (ie. Herring Gull, Common Gull and Black Headed Gull). The Common or Ring Bill Gull, a predominant species found in the metro Toronto area exhibited the highest FC/FS ratio at 585.

The comparison of individual fecal samples from humans and non-humans (Tables 3-8) further demonstrates the variability in indicator bacterial levels and FC/FS ratios that occurs between species and within the same species under varying conditions.

In humans (Table 3) both age and sex of the host seem to have little effect on the ratios. However, consumption of garlic has a profound effect causing reduction in the fecal streptococci levels and subsequent increases in the FC/FS ratios. Some studies suggest that there is little or no relationship between the overall diet of the individual and the altering of the microbial content of the feces (Moore and Holderman, 1975; Finegold et. al., 1975), however, consumption of specific compounds may have an effect (Savage, 1977). Previous dietary studies have reported that vitamin B12 consumption can alter the flora of the intestinal tract (Drasar and Hill, 1974). Garlic may have an effect because it contains the chemical compounds allicin and propene disulfide which are sulfur containing antimicrobials (Branin and Davidson, 1983) and act against gram positive organisms to a greater degree than gram negatives.

The variability in dietary habits, resulting from cultural background, may cause the FC/FS ratios of humans to flucuate from location to location within the Metro Toronto area. For this reason, it may be necessary to examine sanitary sewers in different areas of the city

to obtain the FC/FS ratios representative of each area. The levels and species of bacteria present as well as the FC/FS ratios could be compared with the same data from priority storm sewers at the same locations in order to help detect human fecal pollution.

Differences in the levels of fecal indicators and FC/FS ratios in gull feces from 1984 to 1985 (Tables 4A-4C) may be due to increased stress on the gulls from the use of population control measures (ie. hawks) during the Summer of 1985. When mice were subjected to stress by the removal of bedding material hundred fold increases in their fecal streptococcus levels were noted (Tannock and Savage, 1974). In addition, during a study of the impact of Whistling Swans and Canada Geese on an aquatic ecosystem it was found that stress in the form of fasting or captivity increased FS levels (Hussong, et. al., 1979). Since increases in the concentration of fecal streptococci were evident in gull feces from 1984 to 1985 it may be that gulls are effected in the same way by stressful situations.

Differences that occur between gulls from the Leslie St. Spit and Humber West Park may be due to differences in food types and their availability within the area. The fact that the spit is a nesting site would have some influence on diet during the nesting season, since nesting gulls do not forage over as wide a range as non-nesting gulls (Blokpoel and Tessier, 1985). Different levels of stress may also have an effect.

It is evident that the phenomenon of ratio fluctuation in the feces of Toronto's waterfront birds is more pronounced in certain species (ie. gulls) than in others (ie. geese). This again could be attributed to diet. Gulls are scavengers of human garbage (Mead, 1972; Vernon, 1970 and 1972) whereas geese are primarily grass feeders. The potential effect of a scavenging diet on bacterial fecal flora is provided by a comparison between domestic and wild pigeons (Table 6). Wild pigeons carry much higher levels of E. coli and consequently have higher FC/FS ratios than do domestic pigeons, whose diet consists primarily of commercial feed.

Diet may be the reason that FC/FS ratios fluctuate more in dogs than cats (Table 7 & 8). Dogs tend to be omnivorous and consume a much wider variety of food types than cats who are stricter carnivores.

Using only FC/FS ratios, determined by current procedures, to assess sources of fecal pollution in the Metro Toronto area may present problems due to the similarity to humans feces displayed by some non-human feces as well as the large and diversified populations among both humans and non-humans that exist in this area, and the number of possible input sites for their fecal material. Perhaps the only source currently that can easily be differentiated by FC/FS ratios are livestock because of their very low ratios and the fact that their input sites are more readily determined.

The development of a successful approach to tracing and identifying fecal pollution sources will probably require integrating results from a number of methods. This could include the determination of the concentrations of different bacterial indicators, FC/FS ratios, the identification of bacterial species present, serotyping and/or genotyping combined with a thorough physical observation of an area, good survey design and perhaps the use of tracers, bacterial or otherwise. An ideal place to utilize such a combination approach would be in assessing the presence of illegal or cross connections to storm sewers.

4.1.3 Concentrations of Pathogenic Bacteria in Human and Non-Human Feces (Table 2).

No *Salmonella spp.* or *Campylobacter spp.* were recovered from healthy human sources in this study, however other workers have reported carriage rates of pathogenic bacteria among asymptomatic humans. *Salmonella* as well as *Shigella* have been reported in less than 1% of the population in the United States (Hall and Hauser, 1966; Reller, Ganarosa and Brachman, 1970). *Campylobacter jejuni* has been isolated from 1.3% of asymptomatic persons (Butzler et. al., 1973; Steele and McDermott, 1978). *Pseudomonas aeruginosa* was isolated from humans in this study and has been previously reported in 12% of the population (Sutter et. al, 1967). Since it has been documented that humans can carry pathogenic bacteria in their feces, it is necessary to prevent the

contamination of recreational waters by sanitary sewage from such inputs as combined sewer outflows and illegal sanitary connections.

The recovery of enteric pathogens from non-human sources is extensive. Fecal pathogens (ie. Pseudomonas, Salmonella and Campylobacter) were isolated from almost 100% of the samples tested and in some cases were recovered in high concentrations. There is cause for concern about the health aspects of utilizing recreational waters impacted upon by non-human feces. Referring back to the daily fecal discharge levels presented in Table 19, the potential fecal excretion rate of Pseudomonas aeruginosa from one gull could be as high as 224 organisms/day. The entire gull population on the Leslie Street Spit would have a total output of 3.4 X 107 orgs/day. Survival rates of Pseudomonas aeruginosa in surface waters varies. It is normally not found in unpolluted, non-enriched waters (Hoadley, 1977; Dutka, 1979), but is capable of regrowth in organically enriched, warmer waters (Hoadley et. al., 1968; Wheater, Mara and Oragui, 1979). There is some evidence that it may grow in sewage since the E. coli/Pseudomonas ratios in sanitary sewage are lower than in human feces.

Pseudomonas aeruginosa is the most frequent cause of infection among swimmers (McNeill, 1985). The minimum infective dose (MID) for this pathogen is unknown, but there is evidence of a direct relationship between low

levels of this organism in surface waters and ear infections among swimmers and a control level of between 23 to 30 organisms/100 ml is being considered (Environment Canada, 1980). It is quite possible that warmer polluted waters impacted upon by birds such as gulls could exhibit levels of Pseudomonas far above the suggested control levels. Plasmid mediated antibiotic resistance is common among Pseudomonas strains (Bryan et. al., 1972; Mitsuhashi and Hashimoto, 1975). This tends to make the treatment of otitis externa infections in swimmers more difficult. However, it does become a useful factor when attempting to trace the source of the organism in the environment. Plasmid profiling, antibiotic resistance typing and phage typing has been used successfully in both clinical studies and environmental settings (Seyfried and Flaser, 1977; Palmiteer, personnel communication) to trace the source of Pseudomonas infections. It is possible that these methods could be adapted to differentiate between human and non-human sources of Pseudomonas in surface waters. Pseudomonas lends itself more readily to this type of source determination method because it is easy to recover and exhibits much higher densities in surface waters than do other pathogens (ie. Salmonella and Campylobacter).

The excretion rate of Salmonella spp. from birds such as gulls and to a greater extent ducks, is also high and could reach 6.7×10^3 orgs/day and 31.3 orgs/day per bird. Salmonella has been known to survive for up

to 8 days in surface waters at temperature of 10 to 20°C (Mitchell and Stanzyk, 1975) and a 0.1% survival rate has been documented at 28 days (Dutka and Kwan, 1980). It is quite possible that surface waters receiving daily fecal loading from birds (or other carriers of Salmonella) could also exhibit significant levels of this organism. However, its presence in recreational waters would be less of a concern than would Pseudomonas aeruginosa. The risk of contracting salmonellosis while swimming is almost nil because of the high MID required (1 X 10^5 orgs) (Pitt, 1982). Swimmers would have to swallow a considerable amount of water to obtain this level. Contamination of untreated drinking water supplies is quite possible though, and Salmonella pollution of reservoirs by non-human sources such as gulls has been documented (Benton et. al., 1982; Fennell et. al., 1974; Wilson and Baade, 1959).

Campylobacter presents a more serious problem than Salmonella as an etiological agent of gastroenteritis in surface waters. It is thought to be an important source of acute diarrhea in humans (Blaser and Reller, 1981; Rosef et. al., 1983; Taylor et. al., 1982) and hospital studies have shown that Campylobacter is more often isolated from patients with gastroenteritis than either Salmonella or Shigella (Blaser, Berkowitz and Laforce, 1979). Waterborne transmission of Campylobacter is well documented (Mentzing, 1981; Pearson et. al, 1977; Vogt et. al., 1982; Taylor et. al., 1982). The MID for this organism is quite low

(500 orgs) (Robinson, 1981) which could allow for swimming related outbreaks and the number of possible sources of Campylobacter in the environment (ie. gulls, ducks, geese, pigeons, dogs, cats and livestock) would increase the likelyhood of isolating it from surface waters in the Metro Toronto area. Birds such as wild pigeons can produce heavy loadings of this organism. The fecal excretion rate of Campylobacter from one pigeon could possibly be as high as 294 orgs/day. Since Campylobacter can survive up to 10 days in surface waters at 18 to 26°C (McNeill, 1985) and even longer in colder temperatures (Blaser et. al., 1980; Taylor et. al., 1982) an accumulative effect in waters impacted upon by pigeons or other sources might result.

The contamination of surface waters by organisms such as Campylobacter and Salmonella from livestock feces has been reported in previous studies (Caudon et. al., 1971; Elliott and Ellis, 1977; Dondero et. al., 1977). Cases of waterborne transmission of infections to humans from livestock polluted waters have been documented as well (Diesch, 1970) (Robbins et. al., 1972). It is possible that one cow could excrete up to 14,160 Campylobacter organisms/day and a herd of cattle defecating into surface waters upstream of bathing areas (ie. Clareville Conservation area) could easily create a health hazard situation. Perhaps the most serious aspect of bathing water contamination from livestock feces is not just the numbers of pathogenic

bacteria that can be present but the strains that might be encountered. The incidence of plasmid mediated antibiotic resistant Salmonella and Campylobacter in livestock is high (Blackburn et. al., 1983; Bradbury and Munroe, 1985) and waterborne transmission of pathogens carrying antibiotic resistance factors (R-factors), have caused death in some human patients due to diagnostic problems and failure of adequate response to antibiotic treatment (Blaine et. al., 1977; Gangarosa et. al., 1972).

Although there is a potential for sizable impact on surface waters from non-humans it is difficult to predict the actual extent of contamination by pathogenic bacteria from these sources because the percent populations carrying pathogenic micro-organisms has never been determined for non-humans in the Metro Toronto area. The risk of waterborne transmission of infections to humans is also unknown because both the isolation of pathogens and the epidemiology of swimming at Toronto's beaches have never been undertaken.

4.2 Survival of Fecal Indicators (Table 9).

Detection of pathogens in the water environment depends greatly upon the indicator/pathogen ratio and the persistence of the indicator organism in surface waters in relation to that of the pathogen. The survival rates of indicator bacteria depends upon their resistance to environmental stress.

The method of stressing indicator bacteria used in this study does not totally simulate environmental conditions. However, some factors such as temperature, osmotic pressure, dilution and lack of nutrients are present and would create a stressful environment for the bacteria. Levels of fecal coliforms recovered from all sources after the 24 hour stress period have dropped by 1 to 2 log units and up to 4 log units in some cases. Despite this reduction, there are still substantially higher levels reported and the concentrations would far exceed the level of pathogens present. One interesting result is that the stressed E. coli to Pseudomonas ratio decreased from that found in fresh feces. This is due not only to the decrease in E. coli but also because of the increase in levels of Pseudomonas exhibited after stress. Similar E. coli/Pseudomonas ratios are evident in sanitary sewage where regrowth of Pseudomonas may occur (Michael Young, personal communication).

The concentrations of fecal streptococci also dropped, but there was less change than with fecal coliforms and E. coli. This is due to the differences in die-off rates reported for the two groups. Fecal Coliforms and E. coli have exhibited 90% die-off rates in 2 to 13 days in surface waters (Pitt, 1982; Droste and Gupqupogli, 1982) and complete die-off of these organisms has been reported in less than 14 days (McNeill, 1985). Some fecal coliforms can exhibit regrowth in nutrient rich water environments (McNeill, 1985; McFeters et. al., 1978) but there have been few documented reports on regrowth of E. coli in surface waters (McNeill, 1985) and it is not thought to be a phenomenon that occurs in temperature

zones. Die-off of some species of fecal streptococci in surface waters occurs over a longer period of time. A 90% reduction in FS levels may not be attained even after 30 days in some cases (Pitt, 1982; McNeill, 1985) or take 12 to > 20 days (Mitchell and Slaizyk, 1975). The more resistant gram (+) cell wall and membrane structure of this group may be a factor in their extended survival. However, certain species within the FS group have shorter survival times than E. coli. The die-off rate of S. bovis in surface waters is approximately 90% in 24 hours (McNeill, 1982; Mitchell and Staizyk, 1975).

The effect of the different die-off rates among the two groups greatly influences the fecal coliform to fecal streptococcus ratios obtained after stress. The ratios exhibited in humans and some non-humans decreases so drastically that they all fall below 1 and cannot be differentiated from one another. Dog and gull samples although demonstrating a decrease in ratio, did not exhibit quite as drastic a decrease, and their post stress ratios were still above 4. This may be caused by of the types of bacteria that they carry and will be discussed later on. Ratios from livestock, geese, cats, muskrats and raccoons show either very little change or exhibit increases after stress. This again is more likely due to the types of bacteria carried. This phenomenon of increasing ratios may be used as a method to differentiate sources such as livestock from human sources. A comparison of ratios taken at and downstream of a sampling point where cows have access to the water, would show downstream increases while a similar sampling method used at a site where

human wastes are possibly deposited would exhibit downstream decreases in the FC/FS ratio (McFeters et. al., 1974; Feachem, 1974; Diebel, 1964; Raibaud et.al., 1961). Unfortunately this method could not be used in an area where there are multiple inputs as it would give confusing results.

It is obvious, based upon these findings that the use of FC/FS ratios away from the source would yield poor results and that a sampling program must be conducted as close as possible to outfalls and other input sites.

4.3 Species of Fecal Indicator and Pathogenic Bacteria Isolated from the Feces of Humans and Non-Humans.

4.3.1 Fecal Coliforms (Table 10).

It is well documented that E. coli is the predominant fecal coliform in feces of warm blooded animals (Dufour, 1977; Davis and Matsen, 1974; Geldreich, 1972; Fernstein, 1973; Leclearc, et. al., 1977), although they can be completely absent in 7 to 8% of the human population due to temporary conditions such as illness (Geldreich, 1976). The percent population of E. coli found in this study was always high (Table 10) with some animals exhibiting higher relative levels and some lower than that in humans. Differences were due to the presence of low levels of other coliform bacteria (ie. Klebsiella spp.). Geldreich (1976) has reported Klebsiella spp. carriage in 30 to 40% of the human and animal population he studied. Other studies have

reported 10 to 20% Klebsiella carriage in humans and 30 to 40% in animals (Thom, 1976; Bordner and Carroll, 1972;). Combined data (Table 11) from 1984 (Table 10) and 1985 demonstrate some of the variation possible in the percent recovery of the different coliforms identified in the feces of male and female subjects. The sex of the individual may play a role, however, the distribution of isolates from a composite fecal sample obtained from males and females was dissimilar to either set of individual samples. It is more likely that the differences in the bacterial populations observed result from environmental factors such as diet. The presence of Klebsiella sp. in feces has led to the suggestion that it might have significance as a fecal indicator (Geldreich, 1976), however, it is also found in the environment (McNeill, 1985). Nutrient enriched waters showing little or no evidence of fecal contamination can exhibit high levels of fecal coliform bacteria which can be 50 to > 90% Klebsiella sp. (Huntley et. al., 1976; Dufour and Cabelli, 1976; Vlassoff, 1977). Thus the use of fecal coliform concentrations to assess fecal contamination of effluents and surface waters could be misleading if the FC population is predominantly Klebsiella sp. The presence of E. coli should always be determined in conjunction with standard fecal indicator analyses when first characterizing a survey area or waste effluent to establish the existence of fecal contamination. A higher E. coli target specificity was observed with m-TEC IG (Table 10) resulting in an overall decrease in the percent recovery of non-E.

coli fecal coliforms. The presence of β-D-Glucosidase would appear to be a more consistent characteristic of non-E. coli fecal coliforms than Urease and thus the false positive error is lower with this medium.

The use of E. coli, although excellent for the detection of fecal contamination, would be highly unproductive in the identification of the original source of the feces. Different methods will have to be used for this purpose. Other workers have reported that anaerobic bacteria such as Bifidobacterium spp. could be of value in differentiating human and non-human fecal pollution (Cabelli, 1978). Four species of Bifidobacterium: B. bifidiem, B. adolescentis, B. infantis and B. breve occur exclusively in human feces (McNeil, 1985). The levels of Bifidobacteria in human feces range from 10^9 to 10^{11} per gram (Drasor, 1974; Leven, 1977) which is much higher than the concentration of fecal coliforms. In addition their presence is not affected by either dietary or geographical variation (Drasor, 1974) which would increase their reliability as an indicator.

A further possibility for source differentiation, which is examined in this paper, is by the identification of fecal streptococci present in polluted waters which will be discussed in the following section.

4.3.2 Fecal Streptococci (Tables 12A & 12B)

Enterococci constitute the predominant group of fecal streptococci in humans and most other animals, a fact that has been documented in a number of previous studies (Brown and Gibbons, 1950; Ingram and Burns, 1955; Guthol and Dornmon, 1958; Buttiaux, 1959). Enterococci were recovered from all feces examined as part of this project and constituted greater than 60% of the FS population in all animals except dogs, domestic pigeons, cats and cows in which they represented less than 50% of the isolated FS colonies. Table 19 shows the actual percentage of the presumptive FS colonies isolated from each feces that were enterococci. It can be seen that there is quite a wide range of percent recoveries from 16% in cow feces to 98.4% in goose feces. The low relative recoveries of enterococci are due primarily to higher than average concentrations of S. bovis, Aerococcus sp. and other non-FS microorganisms. The differences between animals are probably due to factors such as physical environment including diet (exposure to fecal pollution) and the conditions within each animals intestinal track. The percent range of enterococci found in feces could act as a rough indication of the source of fecal pollution. For instance, an increasing level of fecal pollution in the direction of an input, i.e. higher FC and EC levels with higher E. coli to fecal coliform ratios, coupled with a continued low fecal coliform/fecal streptococci ratio would be suggestive of fecal pollution without human

input. This type of information would probably be of most use in a rural setting rather than an urban environment where the number and type of fecal inputs could be much higher.

Differences were observed in enterococci population distributions of human and non-human feces. S. faecium is the predominant enterococci species in humans (42%), raccoons (36%) and chickens (29%), however, the second most prevalent enterococci species was S. durans, (22%) in humans, while in raccoons and chickens it was S. faecialis var. liquifaciens (21% and 19% respectively). In ducks, S. faecium and S. faecalis var. liquefaciens were both at approximately 29%. The predominant species in gulls, wild pigeons, muskrats and turkey was S. faecalis var. liquifaciens while goose feces was quite distinctive being the only one with S. faecium var. casseliflavus as the major enterococci. The differences in enterococci population distribution could be used to provide information on possible sources of fecal contamination by observing in-stream population shifts caused by inputs. One factor that still has to be examined is the consistency of these findings in different geographical areas.

TABLE 19: Percentage Enterococci in FS Populations in Feces

Fecal Source	Per Cent Enterococci
Geese	98.4
Turkey	92.5
Wild Pigeons	92.0
Ducks	91.3
Humans	88.6
Muskrats	82.0
Gulls	77.9
Horses	74.6
Raccoons	68.2
Chickens	66.1
Pigs	62.3
Dogs	49.0
Domestic Pigeons	42.8
Cats	38.3
Cows	16.0

Previous studies of the streptococcal flora in humans have resulted in conflicting results. Some workers have found S. faecalis to be the predominant species in humans (Mundt, 1982) while others have recovered higher levels of S. faecium (Orla-Jensen, 1919). Diet has been regarded as a critical factor in influencing the composition of intestinal streptococci and this may explain the inconsistency in the results reported by different studies. Hill et. al. (1971) found that subjects fed on a mainly vegetarian diet carried a higher percentage of S. faecium in their feces while those living on a mixed western diet carried S. faecalis biotypes. Opposite results to this study were obtained by Finegold et. al. (1975) who found that S. faecalis predominated among subjects fed on a mainly vegetarian Japanese diet. There has also been some contradiction as to the major enterococcal species present in animal hosts. Kenner (1978) has suggested that both S. faecium and S. durans predominate in animals while S. faecalis varieties were reportedly found by others (Mundt, 1982; Moussa, 1965).

To compensate for the variation in streptococcal flora which may occur among human and non-human hosts of different geographical areas, it may be necessary to provide a more complex sampling program of impacted surface waters. Such a program would include: 1) detailed site observation to rule out the presence of certain sources of fecal contamination i.e. waterfowl impacting on an area where there are a number of storm

sewer inputs. 2) Sampling and bacterial characterization of sanitary sewage at a given location and/or sampling and bacterial characterization of fecal material from any non-human hosts impacting upon the same area. 3) In line sampling of priority storm sewers as well as sampling of receiving waters directly above and below these inputs. In this way the characteristic streptococcal flora present in sanitary sewage and/or feces may be compared with isolates taken from storm sewers and receiving waters located in the same area to see if any similarities exist while possibly ruling out contributions from sources which may be hard to differentiate from humans.

Characterization of certain physiological and/or biochemical traits within the enterococcal group in general or within certain species belonging to this group may also provide a means of source determination. Distinctions between human and non-human strains of enterococci based on properties other than those used in speciation have been demonstrated. One such property discovered was that human fecal steptococcal isolates could withstand heating at 63°C for 30 minutes and still retain their ability to grow in a medium containing 0.04% potassium tellurite. (Cooper and Ramadan, 1955). Animal strains when tested did not display this property. The occurrence of different biotypes of a particular species in humans and non-humans may also be of use in source determination. Mouloussis (1959) described six fermentative types of

S. faecium but was unable to find any relationship between the fermentative type and its source of isolation. Other workers however, have had success with biochemical characterization of S. faecalis strains. In a 1973 study of S. faecalis isolates obtained from humans, wild animals, plants and insects, Mundt found that over 90% of the 1618 cultures obtained from the non-human sources produced a soft, reduced rennet curd which underwent stratisform digestion in litmus milk or else produced no reaction. Cultures from human origin produced a hard, reduced acid curd which sometimes underwent acid-proteolytic digestion. He thereby concluded that characterization of cultures based on this litmus milk reaction as well as fermentation reactions in melezitose and melibiose could be employed to distinguish between contamination arising from human sources and that from non-human. This procedure would be especially useful for differentiating group D streptococci of non-sanitary significance i.e. plant origin from that of sanitary wastes, as contamination of surface waters by streptococci from environmental sources may yield misleading indications of fecal pollution (Geldreich and Kenner, 1969).

The litmus milk reactions exhibited by human and non-human streptococcal isolates tested in this study (Table 13) differed somewhat from those displayed by Mundts' isolates. The human isolates tested did not exhibit proteolytic digestion of milk, however, 59% of the animal isolates tested also failed to produce this

reaction. The characteristic acid curd of Mundt's human streptococcus isolates was not evident to the same extent in the human isolates tested. Instead many of the isolates from humans (81%) as well as some from animals (44.2%) gave alkaline curd non-proteolytic reactions. This may be an indication that some interaction is occurring between the two groups and animals are acquiring human strains from the polluted environment or that the two sources share some common strains naturally. Over 90% of the environmental isolates of S. faecalis obtained from grass and other plants exhibited the characteristic alkaline and, proteolytic reactions (Seyfried and Harris, 1986 unpublished data) (Appendix B) as was reported by Mundt and therefore non-sanitary isolates could be differentiated from human isolates based on this method. Gulls as well could be differentiated because of the high percentage (97%) of proteinizing strains of S. faecalis found in their feces. However, it would not be advisable to try and differentiate human from non-human sources based on this test alone and other methods should be sought and incorporated into an overall source determination procedure which would include a detailed sampling program as well as the possible development of specific marker bacteria.

4.4 Survival of Fecal Streptococci (Table 15A and 15B)

Fecal streptococci as a group survive well in sewage and surface waters. During sewage treatment, their survival

patterns have more closely resembled that of enteric viruses (Cohen and Shuval, 1973). Regrowth in nutrient rich environments i.e. storm sewers is not thought to be significant (Evans et. al., 1968) although, it has been found to occur in vegetable processing waste water (Mundt et. al., 1966).

A great degree of variation in the survival of different species within the fecal streptococci group exists. Enterococci survive for much longer periods in the aquatic environment than other fecal streptococci i.e. S. bovis and S. equinus (Pitt, 1982; McFetters et. al., 1974; Mitchell and Starzyk, 1975; Geldriech and Kenner, 1969). S. feacalis has been reported to survive up to 28 days in surface waters at 17-18°C (Dutka and Kwan, 1980). It is thought that S. faecium survives for longer periods in warmer polluted waters than S. faecalis but that the two exhibit more closely related survival patterns in colder pristine waters (Dufour, personnel communication, 1985). Both S. bovis and S. equinus exhibit rapid die-off in surface waters. In storm water (20°C) they may survive for a period of only one day (Pitt, 1982). The percent species of fecal streptococci isolated after the 24-hour stress period suggests survival patterns similar to those displayed by these organisms in surface waters. The overall increase in S. faecium from most of the fecal samples confirms the fact that this species survives much better than S. faecalis. Within the S. faecalis group S. faecalis var. liquefaciens showed a more drastic decline in recovery than either S. faecalis var. faecalis or S. feacalis var. zymogenes which suggests that this subspecies may have a more limited survival rate.

There was an overall decrease in recovery of S. bovis from all sources which is to be expected since it has a very short survival time. S. avium also showed a decrease after stress and thus might also exhibit a limited survival in the environment. This trait can be of great use in water quality monitoring since if organisms such as S. bovis and S. avium are recovered at significant levels in surface water sampling then the source of fecal pollution is likely close and of non-human origin. Determining the presence of livestock pollution in impacted surface waters could be accomplished using S. bovis or Aerococcus sp., another organism which exhibits rapid decline under environmental stress.

The end result of subjecting the fecal streptococci populations to stress was to reduce the population heterogeniety both within and between feces. If this is representative of the effects of environmental stress then the ability to distinguish between sources based upon surface water fecal streptococci populations would diminish as one moved away from the source input.

4.5 Occurrence of Salmonella Serotypes in Animal Feces (Table 16)

Salmonella are ubiquitous pathogens of animals and humans and can be recovered from a wide range of hosts. The tolerance of this bacterium for unfavorable environmental conditions is similar to that of saprophytic micro-organisms (Morse et. al., 1976). The Salmonella genus comprises over 1700 serotypes of enteric pathogenic bacteria, a number of which have

been isolated from human infections. Those most commonly reported in Canada are: S. typhimurium, S. saint paul, S. newport, S. enteritidis, S. infantis and S. thompson (Laidley, 1976; Dutka et. al., 1970). U.S. studies have reported: S. typhimurium, S. heidelberg, S. newport, S. infantis and S. enteritidis, S. saint paul, S. typhi, S. derby, S. oranienberg and S. thompson (U.S. Department of Health, Education and Welfare 1969). The top 5 serotypes commonly isolated from humans are: S. typhimurium, S. infantis, S. heidelberg, S. enteritidis and S. newport (U.S. Department Health, Education and Welfare, 1965; Laidley, 1976; Dutka et. al. 1970), and in both countries S. typhimurium continues to be the most frequently isolated causative agent of human salmonellosis (McNeill, 1985; Tacket et. al., 1985; Blackburn et. al., 1983; Vanderpost and Bell, 1975), as well as being the foremost cause of foodborne salmonellosis (Berg and Anderson, 1972).

S. typhimurium is commonly isolated from foodstuffs, animals products, feeds and from surface waters (Dutka et. al., 1970; Reasoner, 1972; Dutka et. al., 1970; Matejovski and Kral, 1970; Morse and Duncan, 1976). It is one of the prevailing types found in animals and birds (Swann et. al., 1967). Berg and Anderson (1972) and MacDonald and Brown (1974) reported S. typhimurium as the type most frequently found in Gulls. Steiniger (1954) found that pigeons and ducks also carried this type.

S. typhimurium was isolated from the feces of gulls, ducks and geese examined in this study (Table 16). It may be that these birds are acquiring this and other Salmonella serotypes

from the environment. Gulls feeding on sewage have been found to carry a variety of serotypes many of which are similar to those found in humans and sewage sludge (McCoy et. al. 1982). In most cases where Salmonella had been isolated, the birds were asymtomatic of disease and were possibly permanent carriers of the organism. A carrier state could easily be established in birds such as gulls due to constant reinfection from sewage ingestion.

Previous studies have indicated that gulls play a role in the epidemiology of Salmonellosis, representing a tremendous reservoir of infection (Berg and Anderson, 1972) and are responsible for the widespread of Salmonella in the environment (Steiniger, 1954). Of the 27 Salmonella serotypes isolated from gulls by Fenlon (1981) 10 were also isolated from livestock and humans living in the same area. These findings would suggest that gulls are vectors in the spread of Salmonella between the two groups. It is quite possible that an environmental cycle exists in which wild birds such as gulls become infected with Salmonella from sewage or polluted surface waters and in turn infect humans and other animals as well as contributing further to environmental pollution by direct defecation into surface waters. (U.S. Department of Health, Education and Welfare, 1969). Other wild animals are commonly infected with Salmonella (Morse et. al., 1976) and may also contribute to the cycle of this organism in the environment. The recovery of S. infantis from raccoons may indicate that these animals are acquiring Salmonella from the environment through the consumption of human garbage and/or contaminated surface waters since S. infantis is a common

serotype isolated from human infections. The degree to which wild animals may act as vectors in the transmission of Salmonellosis depends upon the amount of contact they have with humans, livestock food products and water supplies. It has been documented that rats and other rodents excrete a variety of Salmonella serotypes including S. typhimurium and may continue to excrete these organisms for up to 1 year after initial infection (Welch et. al., 1941). Rodents most likely become infected from the consumption of garbage, offal, sewage and contaminated surface waters and spread infection to humans and livestock through contamination of food and grain.

The control of Salmonella in livestock is very important since it is through consumption of foods prepared from these animals that outbreaks of human Salmonellosis frequently occur (Cherry et. al., 1943; Galton et. al., 1954; Wilson et. al., 1961; Yurack et. al., 1964; Swann et. al., 1967). Livestock represent the largest reservoir of Salmonella in the environment. Of the numerous serotypes that have been isolated, S. typhimurium is the most commonly found type in livestock infections (Edel et. al., 1970; Groves et. al., 1971; Moore et. al., 1976). Two less common serotypes: S. brandenberg and S. senftenberg were isolated from chickens in this study. S. typhimurium was not detected possibly because of the statistically low number of isolates that were serotyped from these birds.

The isolation of Salmonella from the feces of animals impacting in the Toronto area environment including surface waters and the presence of serotypes commonly associated with gas-

troenteritis is cause for concern. The actual impact of pathogens associated with animal fecal pollution deserves further investigation.

4.6 Antibiotic Resistance in Salmonella Serotypes Isolated from Animal Feces (Table 16B)

Organisms of the genus Salmonella are among those most often found to carry antibiotic resistance coded plasmids (Blackburn et. al., 1984). These plasmids are independently duplicating genetic elements separate from bacterial chromosomes and often transferable between bacteria. Resistance plasmids may be extensively shared between Salmonella isolates from animals and humans through contaminated foods, direct contact or via contaminated water (O'Brian et. al., 1982; Alcaide and Garay, 1984). Antibiotic resistant Salmonella have been recovered from cattle, swine and poultry (Blackburn et. al., 1984). It is thought that the addition of prophylactic levels of broad spectrum antibiotics to animal feed selects for resistant Salmonella strains (Olsvik et. al., 1985). Transmissions of these resistant strains from livestock to humans has been documented (Holmberg et. al., 1984; O'Brian et. al., 1982). Selective pressure (antibiotic pressure) can influence the transfer of resistance plasmids between normal intestinal flora such as E. coli and enteric pathogens (Olsvik et. al., 1985), thus creating a situation whereby the animals become carriers of resistant enteric coliform bacilli. Through direct defecation or pastureland runoff these resistant fecal coliforms may be deposited in surface waters and subsequently transmitted to humans.

Grabow et. al., (1975) reported that ingestion of only 10 milliliters of water would be sufficient to obtain a level of resistant E. coli which could colonize the human gut. Resistant plasmids from fecal coliform bacteria can be subsequently transferred back to Salmonella should the host later become infected with this organism.

Antibiotic resistance factors (R-factors) can also be transferred from Salmonella to fecal coliforms in sewage and wastewater and this often occurs during sewage treatment (Bell et. al., 1980; Alcaide and Garay, 1984). Cook (1976) found that fecal coliforms displayed a greater degree of antibiotic resistance than other coliform bacteria and could have a selective advantage in natural waters. The incidence of multiresistant fecal coliform bacteria in city and hospital sewage is high (Bell et. al., 1980; Sturtevant et. al., 1971 and 1969; Grabow and Prozesky, 1973). It is probable that an equally high level of resistant coliform bacteria could be recovered from waters impacted upon by livestock.

Although no resistant Salmonella were recovered from livestock in this study (Table 16B), the Salmonella serotypes isolated from chickens carried a considerable number of plasmids which could be activated at some point in time. A multiresistant Salmonella heidelberg was isolated from gull feces and was able to transfer resistance to a recipient S. typhimurium strain. Multiresistant Salmonella have previously been recovered from gulls. Fenlon (1981) reported that 21 out of 27 Salmonella serotypes isolated from gulls displayed plasmid mediated resistance to chloramphenicol, streptomycin,

neomycin, tetracyline and ampicillin. A widespread of resistant Salmonella in the environment from livestock and wild birds such as gulls could lead to an increased incidence of drug resistant carriers which would in turn make control of Salmonellosis infections more difficult (Luthgen, 1970; Richardson and Watson, 1971).

4.7 Species of Campylobacter in Animal Feces (Tables 17A & B)

The Campylobacter jejunii - C. coli group is now ranked among the leading etiological agents of bacterial diarrhea in humans (Butzler and Skirrow, 1979; Karmali and Fleming, 1979). The majority of cases of human gastroentiritis resulting from Campylobacter infections are due to C. jejunii while C. coli accounts for about 3-5% (Karmali and Skirrow, 1984). Campylobacter laridis belongs to the group of naladixic acid resistant thermophilic Campylobacters. It is a recently described species and has not been associated with human disease except in immunosuppressed individuals (Wachamkin et. al., 1984). C. laridis obtains its name from Gulls (Laridis spp) as these birds are the only hosts from which it is isolated frequently (Benjamin et. al., 1983).

Campylobacter enteritidis has the clinical features of acute gastrointestinal illness. The symptoms of this disease include diarrhea, abdominal pain and fever and can easily be confused with viral gastroenteritis on the basis of symptoms. This illness because it is brief and self limited may go unreported except in severe cases where antibiotic treatment is warranted (Blaser et. al., 1979). Thus it is possible

that swimming associated Campylobacter infections could occur and not be reported.

Previous clinical studies show that poultry, dogs, unpasturized milk, infected children, contaminated food and water are vectors in the spread of C. jejunii (Skirrow, 1977; Hayek and Cruikshank, 1977; Severin, 1978, Lindquist et. al., 1978; Blaser et. al., 1978). Of the Campylobacter isolated from gull feces (Table 17) a high percentage were C. jejunii (52.5%). In a separate study (Table 17B) examining gulls in the Humber West Park area, the carrier rate of C. jejunii among the gulls was reportedly low (5%), however studies conducted in Glasgow, Scotland (Fricker et. al., 1983) documented a much higher incidence of C. jejunii in these birds. It may be that some variation exists between the populations of different geographical areas and that stool positivity peaks during different times of the year as it does with cattle (Blaser et. al., 1984).

Other wild birds such as pigeons may carry C. jejunii (Luechtefeld et. al., 1980; Fenlon, 1981). The recovery of this organism from pigeons was high (69%) which suggests that these birds may also be a vector in the spread of Campylobacter in the environment and contributing to a potential health hazard.

As in the case of Salmonella, livestock have been identified as major reservoirs of Campylobacter jejunii (Blaser et. al., 1984; Luechtefed et. al., 1980) and are important vehicles in the spread of Campylobacter enteritidis in humans by way of

contaminated meat and milk (Blaser et. al., 1984). Spread of infection through livestock contaminated surface waters should also be considered since these animals may excrete large numbers of this organism. C. jejunii was the only Campylobacter species recovered from chickens and cows in the study. Whether or not the animals examined displayed symptoms of enteric illness is unknown. Poultry may harbor this organism without obvious signs of disease (Blaser et. al., 1980). More often with cows, the occurrence of C. jejunii coincides with clinical symptoms of diarrhea. Recovery has been made from healthy cows but usually in much lower numbers (Taylor and Al-Maskat, 1984). Pigs constituted the only source of C. coli in this study. C. coli is commonly present in the feces of normal pigs in small numbers and can be isolated at much higher levels in diarrhetic pigs (Taylor and Al Maskat, 1984). C. jejunii has also been recovered from asymptomatic pigs (Prescott and Bruin-Mosch, 1981). Domestic pets such as dogs and cats have been found to carry C. jejunii although the isolation rate is higher in young animals than in adults. (Bruce et. al., 1980). All of the campylobacter isolated from dogs and cats (Table 19) were identified as C. jejunii. The fecal specimens obtained were all from adult individuals but positive isolation occurred more often from kenneled animals which have a higher incidence of C. jejunii than household animals (Bruce et. al., 1980). Transmission of Campylobacter infections from domestic pets to humans has been known to occur (Coles et. al., 1985), but it is generally regarded that these animals do not represent a major reservoir for the spread of Campylobacter (Blaser et. al., 1984).

Occurrence of antibiotic resistance coded plasmids has been demonstrated within the C. jejunii - C. coli group. Tetracycline, ampicillin and erythromycen resistance in a number of C. jejunii strains has been found (Taylor et. al., 1980 and 1984) as well as resistance to gentamycin (Bradbury and Monroe, 1985). Resistant strains were also found to occur in livestock (Bradbury and Monroe, 1985). Plasmid mediated antibiotic resistance in Campylobacter species could pose the same potential threat as resistance in Salmonella with respect to treatment of infections and transference of resistance factors to other bacteria.

A better understanding of the actual environmental impact of Campylobacter jejunii and the potential health risks to human and animal users of Campylobacter impacted surface waters can be obtained through the examination of waters and sediments where there are known inputs from animal sources.

5.0 CONCLUSIONS

1. E. coli was present in high concentrations and was the predominant fecal coliform in all feces tested. Since no other environmental source has been identified E. coli is an excellent indicator of fecal pollution.

2. The fecal coliform populations present in the intestinal tract of the different animals studied showed insufficient heterogeneity to be used in the identification of sources.

3. The fecal streptococci and Enterococci levels in feces were high, however, problems with the specificity of some media and the potential for other environmental sources suggest these parameters are not as useful in indicating the presence of fecal pollution as E. coli. FS data, in particular Enterococci concentrations, may be valuable when run in conjunction with E. coli.

4. The differences in fecal Enterococci to fecal streptococci levels between animals could provide a rough guide to potential sources. This information would be of most use in areas of minimal inputs e.g. rural and close to inputs.

5. Differences were found in the fecal streptococci populations isolated from humans and different animals. These differences could be exploited in helping to identify sources but again this would have to be done close to inputs.

6. The FC/FS ratios determined in this study indicate that the ratios proposed in 1969 by Geldreich are not applicable to current methods.

7. The FC/FS ratio could be used to help with the identification of original fecal sources in surface waters containing relatively fresh fecal pollution.

8. The application of stress to fecal indicator bacteria altered relative populations and concentrations sufficiently to invalidate the use of population distribution and the FC/FS ratio to identify sources. The results suggest the possibility of significant changes in fecal indicator bacterial populations once they are exposed to the aquatic environment.

9. Bacteria population variability between fecal samples from different members of the same species suggest the potential for significant changes due to such factors as diet and physical environment.

10. Neither Salmonella nor Campylobacter could be isolated from the feces of healthy humans, however, low levels of P. aeruginosa were found. This P. aeruginosa in human feces is not likely to impact on surface waters when fecal material receives proper treatment prior to disposal. If improper or no treatment is received then a potential hazard exists especially if the sewage contains feces from people who are ill or carriers of pathogens.

11. The isolation of pathogenic bacteria from the feces of animals and birds is of definite concern since many of those examined impact directly on the Toronto area environment e.g. gulls, ducks and dogs. The presence of known human pathogens in animal feces is further indication of a potential health hazard.

12. The relatively low ratio of P. aeruginosa to E. coli in feces in comparison to sewage and its ability to grow during some of the stress testing suggests it may be able to grow within Toronto's sewer systems or in nutrient rich polluted surface waters.

13. The Salmonella serotypes recovered from animal sources were often of the same types most commonly isolated from human infections suggesting that animals may be vectors in the spread of Salmonellosis infections to humans and that a possible cycle of this organism exists in the environment.

14. Antibiotic resistant Salmonella isolated from gulls poses a potential threat to human and livestock health as these birds are considered vectors in the spread of infection to both of these groups.

15. The recovery of Campylobacter jejunii from a wide range of animal sources in the Metro Toronto area suggests that this bacterium may be present in the aquatic environment, such as beaches and watersheds of this area.

6.0 RECOMMENDATIONS

1. *E. coli* should be adopted as the parameter of choice for determining the location and assessing the relative impact of fecal contamination in surface waters.

2. The relation of *E. coli*: Fecal coliform ratios to the relative freshness of fecal contamination should be investigated.

3. An epidemiology study of Toronto Beaches should be undertaken to provide information for the development of *E. coli* standards in recreational waters.

4. Concentration of fecal streptococci and enterococci should be used only to assist in the interpretation of fecal pollution data. More data on the significance of enterococci in the aquatic environment is required.

5. The usefulness of FC:FS (EC:ENT) ratios and fecal streptococci species differentiation for source identification should be examined in water, in environments where known fecal impacts occur.

6. The ratios must be appropriate to the enumeration media utilized and only used when sampling occurs close to the source of the fecal pollution. Their use should be restricted until the above field studies have been accomplished, and in any event, may have to be limited to less complex situations i.e. individual point source inputs.

7. The survival times of fecal indicator bacteria and pathogens in Toronto area waters need to be determined. These studies could be combined with transport studies to investigate transport time of bacteria in surface waters and would provide more information on the potential impact of sources.

8. Toronto area waters impacted by fecal pollution should be examined for human enteric bacterial pathogens and the pathogens isolated should be tested for antibiotic resistance.

9. An investigation of the percent population of animals carrying pathogenic bacteria such as Salmonella and Campylobacter, concentrating on those species of animals which have a major impact on the Toronto waterfront area, i.e. gulls, should be conducted.

10. The ability of P. aeruginosa to grow in sewers and nutrient enriched surface waters needs to be assessed.

11. Surveys to identify sources and locate fecal pollution inputs need to concentrate their efforts around individual inputs after they are located. This could be accomplished in two stages:
    1) Overall pollution surveys
    2) Intensive surveys in restricted geographical areas.

12. Determine bacterial populations and ratios in a number of residential sanitary sewers to determine the consistency of the human fecal bacterial pollution results obtained in this study.

13. Mount an investigation of priority storm sewers, sanitary sewers and storm water runoff serving the same geographical areas to determine their specific bacterial populations.

14. Isolates from the above study should be analyzed to determine phenotypes, serotypes and genotypes that could be used as specific markers of human fecal pollution for the rapid detection of sanitary wastes in storm and surface waters.

15. Since none of the findings to date provide conclusive proof of human versus non-human fecal pollution with respect to levels and species of indicator bacteria present, investigation of new fecal parameters should continue. Bifidobacteria and Clostridia have been suggested as potential indicators.

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8.0 APPENDIX A:

A - 1 Relationships of Enterococci, Group D Streptococci and Viridans Streptococci.

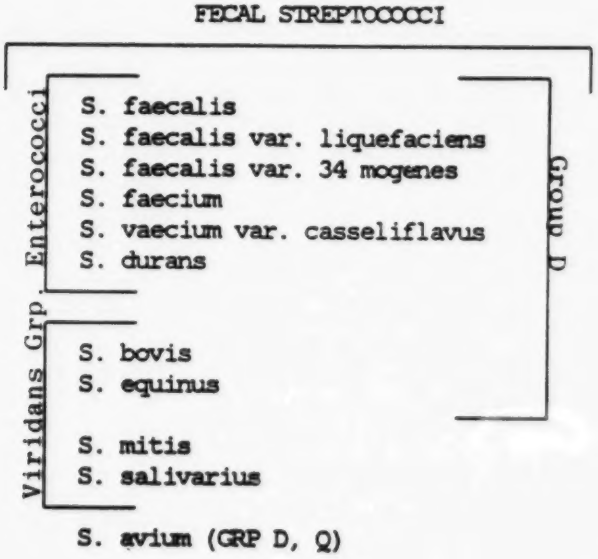
A - 2 Scheme for the Identification of Streptococcus Isolates Picked From m-Enterococcus or Other Similar Agars.

A - 3 References used to develop Identification Scheme.

APPENDIX A - 1

Relationships of Enterococci, Group D Streptococci and Fecal Streptococci

Modified from Hartman.



Ref: Hartman et. al., Indicator Organisms - A Review Taxonomy of the fecal streptococci (Int. Journ. Syt. Bact. V 16-2, April, 1966, pp. 197-221

APPENDIX A - 2: Identification Scheme for Feacal Streptococci

1) Pick isolated colonies from m-Enterococcus or other similar agar.
2) Transfer the growth to blood agar (5% rabbit's blood) and streak out for isolated colonies. Incubate the blood plates at 35°C for 24 hours.
3) Examine incubated blood plates for purity and growth of the culture. Select one isolated colony from pure cultures only and prepare a reservoir of growth on Brain-Heart Infusion agar (BHI). Incubate the BHI plates at 35°C for 24 hours.
4) After incubation, use reservoir of growth on BHI agar to check the Gram reaction (3% KOH method) and catalase reaction of the isolate.
5) If the isolate is Gram positive, catalase negative, then use the growth on BHI agar to inoculate the following physiological tests:

TEST	INCUBATION PERIOD
1) Bile Esculin Agar	1 to 3 Days
2) Todd Hewitt Broth for growth at 10°C	5 Days
3) Todd Hewitt Broth for growth at 45°C	3 Days
4) 6.5% NaCl (in Heart Infusion Broth)	3 Days
5) Thornley's Arginine dihydrolase medium	3 Days
6) 2% soluble starch (in blood agar base)	5 Days
7) Tellurite Agar (0.04% Potassium Tellurite)	5 Days
8) 1.1% Arabinose (in heart infusion broth)	3 to 5 Days
9) Gelatin (12% in heart infusion broth)	5 Days
10) Pyruvate Broth	1 Day
11) 1% Mannitol (in heart infusion broth)	3 to 5 Days

TEST	INCUBATION PERIOD
12) 1% Lactose (in heart infusion broth)	3 to 5 Days
13) 1% Sorbose (in heart infusion broth)	3 to 5 Days
14) Methylene Blue Milk (0.1% Methylene Blue in Skim Milk Broth)	1 Day

The above series of test will allow for separation of Enterococcus species from other faecal streptococci and from non-faecal streptococci. (See Table #1) Potential S. bovis, S. equinus and S. avium isolates must be tested serologically for the group D Antigen (See Serological Testing Method) before their identification can be confirmed, since they show biochemical reactions similar to the Viridans streptococci group. Varients of S. faecalis must be tested for their haemolysis reactions under anaerobic conditions on 5% rabbit's blood.

TABLE 1: Some Physiological Reactions of the Fecal Streptococci and Some Physiologically Similar Viridans Streptococci and Aerococci Useful for Differentiation.

	Bile Esculin	Growth @ 10°C	Growth @ 45°C	Growth in 65% NaCl	Arginine	Starch	Tellurite	Arabinose	Gelatin	Tetrazolium	Haemolysis	Yellow Pigment	Methylene Blue Milk	Pyruvate	Mannitol	Lactose	Sorbose	Sero Group
S. faecalis	+	+	+	+	+	-/+	+	-	-	+	α/γ	-	+	+	+	+	-	D
S. faecalis V. liquefaciens	+	+	+	+	+	-	+	-	+	+	α/γ	-	+	+	+	+	-	D
S. faecalis V. zymogenes	+	+	+	+	+	-	-	+	V	+		-	+	+	+	+	-	D
S. faecium	+	+	+	+	+	-	-	+	-	+		-	+	+	+	+	-	D
S. faecium V. casseliflavus	+	+	+	+	+	-	V	-	-	+		-	+	-	+	+	-	D
S. durans	+	+	+	+	+	-	-	+	-	V		-	+	-	+	+	-	D
S. avium	+	+	+	+	-	-	-	+	-	V		-	+	+	+	+	+	Q/D
S. bovis	+	-	+	-	-	+	-	-	-	V		-	V	-	+	+	-	D
S. bovis variant	+	-	+	-	-	-	-	-	-	V		-	V	-	-	+	-	D
S. equinus	+	-	+	-	-	-	-	-	-	-		-	-	-	-	-	-	D
S. mutans	-/+	-	+/-	-	-	-									+	+		E/NG
S. mg intermedius	-/+	-	+/-	-	+/-	+/-									-	+		F/NG
S. salivarius	-(d)	-/+	+/-	-	-	-									-	+		K/NG
S. sanguis I	-(d)	-	+/-	-	+/-	+/-									-	+		F/NG
Aerococcus sp.	+/-	+	-	+	-													

V = Variable D = Some May Differ NG = Non Groupable

NOTE: 1) Some S. bovis (Starch-'ve) variants cannot be distinguished from certain viridans streptococci (ie. S. mg intermedius and S. Sanguis I) except by serology.

2) The above reaction patterns are the most common, variants for any particular test may occur.

STREPTOCOCCUS - SEROLOGICAL GROUPING

Principle of Test:

The majority of streptococcus species possess group-specific antigens which are usually carbohydrate structural components of the cell wall. These antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera. There are a number of ways to extract these antigens from the cell wall including 1) Hot HCL extraction 2) Hot formamide extraction 3) Autoclave extraction 4) Sonication and 5) enzyme extraction. Each extraction method has certain advantages and disadvantages. Generally the autoclave extraction method and the enzyme extraction method are simple yet reliable procedures for all groups including Group D. Some Group D streptococcus species (ie. S. bovis, S. equinus and S. avium), however, contain relatively small amounts of this antigen and these may require more severe extraction procedures (ie. sonication).

The antigen-antisera precipitation reactions can be performed in various ways including 1) Capillary precipitin test 2) Slide agglutination reaction 3) Electrophoretic methods.

Probably the simplest method to employ is the slide agglutination procedure whereby group-specific antibody coated latex particles are reacted with the antigen extract.

There are commercially prepared kits available which provide the enzyme for an enzyme extraction, a reaction slide and antibody coated latex particles for various serogroups (generally groups A, B, C, D, F and G). These latex particles can also be reacted with extract from any other extraction procedure.

Methods

Enzyme Extraction - latex agglutination (Commercially available Kit).

1) Quality Control - The kit contains a vial of polyvalent antisera. Mix one drop of each latex compound into one drop of antisera to ensure that each latex compound reacts appropriately.
2) Rehydrate the lyophilized extraction enzyme.
3) Cells for the extraction procedure for the unknowns can be taken from a plate or broth culture.
   a) Plate - Sweep a light loopful of growth from a 24 hour blood plate and emulsify in 0.4mL Extraction enzyme.

OR

   b) Broth - take one drop of a 24 hour Brain heart infusion + 1% dextrose broth culture and add to 0.4mL Extraction enzyme.
4) Incubate the extraction enzyme cell mixture one hour at 37°C (water bath).
5) Dispense one drop of the appropriate latex per corresponding circle on the agglutination slide.
6) Add one drop of the incubated extract to each latex drop.
7) Mix until smooth and milky using a separate applicator stick for each circle.
8) Gently rock and rotate the slide until a reaction occurs in one of the circles (usually within one to two minutes).
9) Record which group antigen if any is present.

Autoclave Extraction of Group Antigen

1) Grow pure isolate in Brain heart infusion broth plus 1% dextrose (final concentration) for 24 hour sat 35°C, in a 16 x 100 mm screw cap tube, 8mL broth per tube (tube must be suitable for centrifugation).
2) After incubation centrifuge broth culture at 3000 rpm for 10 minutes, to pack cells.
3) Decant liquid into a disinfectant and retain cells.
4) Add approximately 5mL of aqueous 0.85% NaCl solution (physiological saline) to the tube and resuspend cells.
5) Centrifuge the cells a second time at 3000 rpm for 10 minutes, to pack cells.
6) Decant liquid into a disinfectant and retain cells.
7) Add 2-3mL physiological saline to the tube and resuspend the cells.
8) Autoclave the tube 15 minutes, 15 psi (121°C).
9) After autoclaving, allow to cool and centrifuge the tube at 3000 rpm, 5 minutes to pack cells.
10) Once the cells are packed the liquid (extract) phase can be used to perform the serological testing (see latex method).

APPENDIX A - 3: References Used to Develop Scheme for Fecal Streptococcus Identification.

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9.0 APPENDIX B

B-1 Occurrence of Group D Streptococci on plants obtained from the Humber River/Black Creek Area.

B-2 Litmus Milk Reactions of Streptococcus faecalis obtained from plants.

APPENDIX B-1 Occurrence of Group D Streptococci on plants obtained from the Humber River/Black Creek Area.

SOURCE	% OCCURRENCE								
	SFM	SFC	SFF	SFL	SFZ	SD	SB	SBV	TOTAL ISOLATES
HUMBER RIVER (AT ALBION RD.)	37.3	52.9		3.9		5.9			51
BLACK CREEK (HYDE AVE. CSO)	51.6					48.4			31
HUMBER RIVER (AT JAMES GARDENS)	23.4	10.6		12.8		47.9		5.3	94
HUMBER RIVER (NEAR BLOOR ST.)	61.9	4.8		14.3		19.0			42

ABBREVIATIONS

SFM	S. faecium		
SFC	S. faecium	var.	casseliflavus
SFF	S. faecalis	var.	faecalis
SFL	S. faecalis	var.	liquefaciens
SFZ	S. faecalis	var.	zymogenes
SD	S. durans		
SB	S. bovis		
SBV	S. bovis	varient	

APPENDIX B-2 Litmus Milk Reactions of Streptococcus Faecalis obtained from plants.

	PERCENTAGE LITMUS MILK REACTIONS				
# OF ISOLATES	REDUCTION ONLY	REDUCTION ALKALINE CURD	REDUCTION ACID CURD	ALKALINE PEPTONIZATION	ACID PROTEDYSIS
20	-	50	-	95.0	-